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FREDERICK DEFOREST HEALD, 1872-1954

George W. Fischer

Frederick DeForest Heald, plant pathologist, mycologist, botanist, and in all a scientist, died in Spokane, Washington, April 24, 1954, at the age of 82. He had been Professor Emeritus since his retirement in 1941 from the position of Plant Pathologist and Professor of Plant Pathology in the Agricultural Experiment Station and the College of Agriculture and Head of the Department, at the State College of Washington. He retired with an enviable record of professional achievement which had long since won him international renown.

Dr. Heald was born on July 23, 1872, at Midland City, Michigan, the son of Henry Francis Heald and Hettie Charles Heald. He graduated from the University of Wisconsin with the B.S. degree in 1894 and with the M.S. degree in 1896. Following a year of graduate study in plant physiology and plant pathology at the University of Leipzig, he received the Ph.D. degree in 1897.

His first professional position (1897-1903) was that of Professor of Biology at Parsons College, Fairfield, Iowa. In 1903 he was appointed Adjutant Professor of Plant Physiology at the University of Nebraska and in 1905 Associate Professor of Botany and Botanist of the Nebraska Agricultural Experiment Station. In 1906 he was promoted to the rank of Professor of Agricultural Botany. In 1908, Dr. Heald moved to the University of Texas as Head of the School of Botany. In 1912 he went to work for the State of Pennsylvania and the U. S. Department of Agriculture as Plant Pathologist, investigating biological factors involved in the serious chestnut blight epiphytotic. His contributions to the knowledge of the life history of the pathogen and means of spread of the disease undoubtedly led to his appointment in 1915 as Professor of Plant Pathology at the State College of Washington and Plant Pathologist in the Washington Agricultural Experiment Station.

By now Dr. Heald was so thoroughly convinced of the importance of the field of plant pathology that he was able to persuade the college administration to create a separate department. This was accomplished in 1918 and it is entirely logical that this tremendously energetic and courageous man was installed as head of the new department.

In 1899, Dr. Heald married Nellie Townley. Much of his outstanding professional development can be attributed to the constructive and balancing influence of this brilliant woman. From this marriage came three children: Doris Heald Tonge, Henry Townley Heald, and Marion Heald Shebasta. Mrs. Heald died in 1939. In 1942 he married Charlotte Chamberlin. She and all three children survive him.

Dr. Heald's first contribution in plant pathology came in 1905 and from this point on, his papers dealt

FREDERICK DEFOREST HEALD, 1872-1954

almost exclusively with plant pathology and mycology. After 1915 his publications reflected 2 predominant fields of interest: cereal smuts, and storage decays of pears and apples. In 1926 the first of two editions of his *Manual of Plant Diseases* appeared. This book served a very great need and quickly became the plant pathologist's "Bible" because it was a scholarly and exhaustive reference book for so many plant diseases. He later wrote *Introduction to Plant Pathology*, a shorter version adapted to single-term classes. In 1941 he collaborated with C. S. Holton in the publication of *Bunt or Stinking Smut of Wheat (A World Problem)*. Dr. Heald is author or co-author of more than 120 scientific papers, bulletins, etc., covering a period of 45 years. He also served as special editor for the second edition of Webster's International Dictionary. Dr. Heald was a member of various scientific and professional societies. He was President of the American Microscopical Society in 1912 and of the American Phytopathological Society in 1932. He was an associate editor of PHYTOPATHOLOGY for 8 years.

Dr. Heald's influence has been pronounced professionally through accomplishments in research, in teaching, and in scholarly writing. Also, the personality and drive of the man himself has had no small influence on his students and associates. His students are impressively in agreement on his ability as a teacher. They are well distributed in responsible positions and their performance has attested the high quality of the teaching and direction they received.

PHYTOPATHOLOGY for July (45:351-408) was issued July 18, 1955

THE USE OF PATHOLOGICAL TECHNIQUES TO DISTINGUISH GENETICALLY DIFFERENT SOURCES OF RESISTANCE TO CROWN RUST OF OATS [1]

M. D. Simons[2]

SUMMARY

The reactions of 13 resistant varieties and strains of oats to different races of crown rust under different environmental conditions were observed in order to study the possibility of distinguishing between previously known and new genetically distinct sources of resistance by the use of certain pathological techniques.

The resistance to race 202 of seedlings of Landhafer and P.I. numbers 185663, 185785, and 198227, grown in sand culture furnished with a nutrient solution high in nitrogen and held at high temperatures, was broken down sufficiently to differentiate these selections as a group distinct from all others.

Reactions to 6 races of crown rust indicated that the genes involved in the crown rust resistance of Silva Selection and of P.I. numbers 186603 and 189624 differ from those of other varieties and strains studied, and probably represent a higher degree of resistance. The use of a number of different races of crown rust also indicated that P.I. 186610, similar to Victoria in many respects, is resistant to some of the common races that attack Victoria.

Certain races appeared to be more effective than others in differentiating varieties by the detached-leaf culture technique.

Maleic hydrazide 30, at a concentration of 0.35 per cent, completely suppressed crown rust development on all varieties and strains tested except Victoria and P.I. 186610, in which it induced a susceptible reaction. DDT had little or no effect on the reactions of any of the varieties or selections to 5 races of crown rust.

The most important means of controlling crown rust of oats, caused by *Puccinia coronata* Cda. var. *avenae* Fraser & Led., is to breed resistant varieties. The success of this method depends on the availability of good sources of resistance. At present, there are only a few known genetically different sources of resistance to the common North American races of crown rust. New genes for resistance would be highly desirable.

When material of unknown parentage is found to be resistant to the common races under normal conditions, the investigator is immediately faced with the problem of determining whether the genes involved differ from those of previously known sources of resistance. Genetic studies provide the surest answer, but require much time and labor, and the task becomes increasingly difficult as more resistant varieties are discovered. Morphological comparisons of the new variety with the known varieties or selections are sometimes helpful, but are not conclusive. This investigation was undertaken to study the practical application of certain pathological approaches to the problem of distinguishing among different gene complexes involved in resistance to crown rust.

Peturson (16), Murphy (13), and Simons (17) independently studied the reactions of various varieties of oats in the seedling stage to different races of crown rust at different temperatures. Their data indicated that the reactions of certain varieties were not greatly influenced by temperature, regardless of the race that was used. The reactions of other varieties to certain races, however, varied considerably at different temperatures. In general, where reactions varied with temperature, the more susceptible reactions were observed at the higher temperatures.

Pantanelli (15) and Gassner and Hassebrauk (7) reported that certain varieties of oats when supplied with nutrient solutions high in nitrogen were somewhat more susceptible to crown rust than when the solutions contained moderate or low quantities of nitrogen. Another possible means of distinguishing differences in resistance has been suggested by Browning (2, 3), who showed that certain varieties of oats normally resistant to a given race of crown rust were susceptible to that race when inoculated leaves were detached and supplied with 2 per cent glucose or sucrose solutions.

Livingston (12) reported that the diethanolamine salt of maleic hydrazide, sprayed on wheat at a concentration of 0.1 per cent, resulted in an increase in the size of pustules of leaf rust. Bromfield and Peet (1) showed that wheat seedlings treated with maleic hydrazide were consistently more susceptible to stem rust than were nontreated seedlings. The insecticide DDT (dichloro diphenyl trichloroethane), when applied as a spray to the leaves of Khapli emmer, caused this variety to show susceptibility to certain races to which it is normally resistant (1, 8, 10).

MATERIALS AND METHODS.—The varieties of oats used in this study included 5 previously known sources of resistance (Landhafer, Santa Fe, Trispernia, Bondvic, and Victoria). Lines chosen as possibly representing new sources of resistance (Silva Selection, and P.I. numbers 174513, 185663, 185785, 186603, 186610, 189624, and 198227) were selected on the basis of preliminary field and greenhouse tests made in

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Cooperative investigation between the Field Crops Research Branch, Agricultural Research Service, U.S. Department of Agriculture, and the Iowa Agricultural Experiment Station. Journal Paper No. J-2665 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 1176.

[2] Plant Pathologist, Field Crops Research Branch, Agricultural Research Service, U.S. Department of Agriculture.

1952. P.I. numbers 174513 and 198227 have been reported by other investigators to be resistant under field conditions (4, 9). Cultures of the 6 races of crown rust used were designated as follows: race 202, stock culture no. 2; race 203, stock culture no. 7; race 205, stock culture no. 1; race 216, stock culture no. 16; race 258, stock culture no. 8; and race 263, stock culture no. 18. Each of the first 5 cultures was increased from a single uredium. The culture of race 263 was increased from a field collection. Techniques of inoculation, isolation, and increase of crown rust races, and notation of reaction type were similar to those described by Murphy (13), and Finkner, Atkins, and Murphy (5).

REACTIONS TO VARIOUS RACES OF CROWN RUST UNDER NORMAL CONDITIONS.—The varieties and selections used in this study were all resistant to the common crown rust race 202 under normal greenhouse conditions, where the plants were grown in ordinary potting soil at temperatures of 70°–75°F during the period from October to March. However, the term "resistant" simply means that few or no spores are produced, and includes a number of readily distingiushable reaction types.

The use of additional races, particularly rare or unusual races, increases the chance of distinguishing among different genes for resistance, by production of differing types of resistant reactions. There is also the possibility that some of the selections under consideration will be clearly separated from the others by being susceptible to certain races not previously used for testing them.

The 13 varieties and selections were tested for reaction to 6 races of crown rust (Table 1). The variety Markton, susceptible to all the races used, was included as a check. Three selections, Silva Selection, P.I. 186603, and P.I. 189624, showed no macroscopic evidence of infection when tested for reaction to race 202. Six varieties and selections, Landhafer, Santa Fe, P.I. 174513, P.I. 185663, P.I. 185785, and P.I. 198227, reacted with a light flecking to this race. Trispernia and Bondvic were a little less resistant, supporting a few small uredia. Victoria and P.I. 186610 produced 2-type uredia associated with a type of necrosis characteristic of Victoria.

The reactions of all varieties and selections to race 203 were similar to their reactions to race 202. The reactions to race 205 resembled those of races 202 and 203, except that Victoria and P.I. 186610 were apparently immune from race 205.

Races 216 and 258 vigorously parasitized Victoria but not P.I. 186610, thus serving to distinguish between these 2 selections which reacted similarly to all other races.

Only 6 varieties and selections, Victoria, Silva Selection, P.I. 174513, P.I. 186603, P.I. 186610, and P.I. 189624, were resistant to race 263. These 6 were not highly resistant, but exhibited 2-type uredia and rather severe local necrosis resembling the reaction of Victoria to the common races of crown rust.

TABLE 1. *Average reactions*[a] *of 14 varieties and strains of oats to 6 races of crown rust*

Variety or selection[b]	Race 202	Race 203	Race 205	Race 216	Race 258	Race 263
Landhafer						
C.I. 3522	0	0	0	0	0	4
P.I. 185663	0	0	0	0	0	4
P.I. 185785	0	0	0	0	0	4
P.I. 198227	0	0	0	0	0	4
Victoria						
C.I. 2401	2	1	I	4	3	2
P.I. 186610	2	1	I	0	1	2
Santa Fe						
C.I. 4519	0	0	0	0	0	4
Trispernia						
C.I. 4009	0, 1	0, 1	0	0, 1	0, 1	4
Bondvic						
C.I. 5401	0, 1	0, 1	0, 1	0, 1	0, 1	4
Silva Sel.	I	I	I	I	I	2
P.I. 186603	I	I	I	I	I	2
P.I. 189624	I	I	I	I	I	2
P.I. 174513	0	0	0	0	0	2
Markton						
C.I. 2053	4	4	4	4	4	4

[a] "I" indicates no macroscopic evidence of infection; "0," necrosis or chlorosis but no uredia; "1," necrotic or chlorotic areas, some of which contain small uredia; "2," necrotic or chlorotic areas, most of which contain small to mid-size uredia; "3," chlorotic areas surrounding abundant mid-sized uredia, no necrosis; and "4," no necrosis or chlorosis, uredia large and abundant.

[b] C.I. refers to accession number of Cereal Crops Section, A.R.S., U. S. Dept. of Agriculture. P.I. refers to accession number of Plant Introduction Section, A.R.S., U. S. Dept. of Agriculture.

EFFECT OF A HIGH LEVEL OF NITROGEN NUTRITION AT HIGH TEMPERATURE.—Preliminary investigations showed that either high temperature or a high level of nitrogen nutrition increased the susceptibility of certain varieties of oats to crown rust. This increase in susceptibility was more pronounced when high temperature and high nitrogen level were combined. Plants of the 13 varieties used in the present investigation, grown at about 85°F in clean quartz sand and supplied with essentially the same high nitrogen nutrient solution used by Gassner and Hassebrauk (7), were tested for reaction to race 202. The test was carried out once in December and once in May, with no artificial light used either time.

None of the treated varieties or selections showed any appreciable increase in susceptibility over the checks grown in soil at 70°–75°F under the long-day conditions of May. In the December test, Landhafer was more susceptible with high nitrogen and high temperature than it was under normal conditions. This variety exhibited a uniform 0-type reaction under normal conditions and a mesothetic reaction with individual lesions ranging from 0- to 3-type at the high temperature. P.I. numbers 185663, 185785, and 198227 were similar to Landhafer in all respects under both sets of environmental conditions. None of the other varieties or new selections showed different reactions as compared with their untreated checks.

REACTION OF DETACHED LEAVES.—Material for testing reactions of detached leaves to 6 races of crown rust was obtained by growing plants in soil until the first leaf was about 3 in. long. The plants were then inoculated in the usual manner. In several separate experiments leaves were detached 2 and 4 days after inoculation, and placed in test tubes, each tube containing about 3 ml of 2 per cent sucrose solution.

The results were somewhat erratic, and often not clear-cut. It was noted for example, that detached leaves of a given variety were seldom heavily infected with a race to which the variety was normally resistant. Usually these leaves would show only a few uredia, and the results were not always confirmed in subsequent tests. In contrast, detached leaves infected with a race to which they were normally susceptible consistently showed very heavy infection of 4-type uredia.

In spite of these difficulties, it was possible to make certain general observations. Detached leaves often showed greater susceptibility than nondetached leaves of the same variety or selection. When all 6 races were considered, this trend was more pronounced in varieties or selections normally moderately resistant to a given race than in those with a higher type of resistance. Thus detached leaves of the selections that were normally moderately resistant to race 263 were susceptible. Conversely, no uredia were seen on detached leaves of Silva Selection, P.I. 186603, or P.I. 189624 that had been inoculated with the other 5 races, from which they were normally immune.

The races used did not appear to be equal in their ability to induce susceptible reactions in detached leaves of normally resistant varieties. Almost no uredia were seen on detached leaves of any of the varieties or selections inoculated with race 205. At the other end of the scale, race 216 induced susceptible reactions rather consistently in certain normally resistant varieties and selections.

EFFECT OF MALEIC HYDRAZIDE.—Maleic hydrazide 30 (a solution of MH as diethanolamine salt containing 30 per cent active MH by weight) at a concentration of 0.35 per cent was sprayed on seedling plants of the 14 varieties and selections being studied. Two days later, the plants were inoculated with race 202. Most of the varieties and selections, including the highly susceptible Markton, that were sprayed with maleic hydrazide never developed any macroscopic symptoms of infection. Victoria and P.I. 186610 produced a few medium-sized uredia surrounded by green tissue with no sign of chlorosis or necrosis. Nontreated checks of both Victoria and P.I. 186610 were heavily infected with small uredia, each uredium being situated in the center of a small necrotic area. Further tests with maleic hydrazide involving different concentrations, lower and higher temperatures, and different races of crown rust were carried out. These further tests substantiated the results just described in some cases, but did not add any information of value on the other varieties.

EFFECT OF DDT.—The insecticide DDT, prepared and applied in essentially the same manner as described by Johnson (10), did not increase the susceptiblity of any of the 14 varieties and strains of oats inoculated with 5 races of crown rust (202, 203, 205, 216, 258). The treatment, in fact, slightly reduced the susceptibility of the normally highly susceptible variety Markton.

REACTIONS TO HELMINTHOSPORIUM VICTORIAE MEEHAN & MURPHY.—In view of the well-known relationship of the Victoria type of crown rust resistance and susceptibility to *H. victoriae* (11, 14), it might be suspected that any variety susceptible to *H. victoriae* might carry the same gene for crown rust resistance that is found in Victoria. The 6 varieties that were resistant to race 263 (Victoria, Silva Selection, P.I. 174513, P.I. 186603, P.I. 186610, and P.I. 189624) were susceptible to *H. victoriae*. The remaining 8 varieties were resistant to *H. victoriae* but were susceptible to crown rust race 263.

DISCUSSION.—It was apparent that the 13 resistant varieties and selections that were studied fell into several distinct groups as based on their reactions under different conditions. One of the most interesting of these groups included Silva Selection, P.I. 186603, and P.I. 189624. Under normal conditions, these 3 selections developed no macroscopic symptoms and thus appeared to be immune from the common races of crown rust. This indicates that they have a distinctly higher degree of resistance than any of the named varieties. They were moderately resistant to race 263, their reaction to this race resembling the reactions of Victoria and P.I. 186610. Also, like Victoria and P.I. 186610, they were susceptible to *Helminthosporium victoriae*. These facts, in view of the epistatic nature of the Victoria gene insofar as reaction to *H. victoriae* is concerned (6), suggest that the crown rust resistance of these 3 selections may be attributed to the Victoria gene in combination with other genes, possibly including those of Landhafer or Santa Fe.

P.I. 174513 was similar to the 3 selections just discussed except that it showed a flecking rather than an immune reaction to the common races of crown rust.

Another distinct reaction group, comprised of Landhafer, P.I. 185663, P.I. 185785, and P.I. 198227, also was clearly distinguished from all other varieties and selections by a partial but unmistakable breakdown of resistance when supplied with a nutrient solution high in nitrogen at high temperatures.

P.I. 186610, which like Victoria is susceptible to *H. victoriae*, also showed typical "Victoria type" resistant reactions to races 202, 203, 205, and 263. P. I. 186610, however, also was resistant to races 216 and 258, which attack Victoria. Because of the resistance of P.I. 186610 to the common races attacking Victoria, and the fact that it otherwise closely resembles Victoria, this selection may be useful as a source of crown rust resistance in areas where considerable acreages

of varieties susceptible to *H. victoriae*, are grown successfully.

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LITERATURE CITED

1. BROMFIELD, K. R., AND C. E. PEET. 1954. Chemical modification of rust reaction of seedling wheat plants infected with Puccinia graminis tritici. Phytopathology **44**: 483.
2. BROWNING, J. A. 1953. Studies in the physiology of obligate parasitism in the cereal rusts. Ph.D. thesis. Cornell University, Ithaca, New York. 84 p.
3. BROWNING, J. A. 1954. Breakdown of rust resistance in detached leaves of normally resistant oat varieties. (Abs.) Phytopathology **44**: 483.
4. EARHART, R. W., AND J. G. MOSEMAN. 1953. Possible desirable germplasm in new oat introductions grown in Florida and North Carolina in 1952-53. U. S. Dept. Agr. Pl. Dis. Rptr. **37**: 597-598.
5. FINKNER, R. E., R. E. ATKINS, AND H. C. MURPHY. 1953. Inoculation techniques for crown rust of oats. Agron. Jour. **45**: 630-631.
6. FINKNER, V. C. 1953. Inheritance of susceptibility to Helminthosporium victoriae in crosses involving Victoria and other crown rust resistant oat varieties. Agron. Jour. **45**: 404-406.
7. GASSNER, G., AND K. HASSEBRAUK. 1934. Der Einfluss der Mineralsalzernährung auf das Anfälligkeitsverhalten der zur Rassenbestimmung von Getreiderosten dienenden Standardsortimente. Phytopath. Ztschr. **7**: 63-72.
8. HOTSON, H. H. 1953. Some chemotherapeutic agents for wheat stem rust. Phytopathology **43**: 659-662.
9. IVANOFF, S. S. 1953. 1952 National Oat Newsletter (Sponsored by the National Oat Conference) **3**: 48-49.
10. JOHNSON, T. 1946. The effect of DDT on the stem rust reaction of Khapli wheat. Canad. Jour. Res. Sec. C **24**: 23-25.
11. LITZENBERGER, S. C. 1949. Inheritance of resistance to specific races of crown and stem rust, to Helminthosporium blight, and of certain agronomic characters of oats. Iowa Agr. Exp. Sta. Res. Bul. 370: 454-496.
12. LIVINGSTON, J. E. 1953. The control of leaf and stem rust of wheat with chemotherapeutants. Phytopathology **43**: 496-499.
13. MURPHY, H. C. 1953. Physiologic specialization in Puccinia coronata avenae. U. S. Dept. Agr. Tech. Bul. 433, 48 p.
14. MURPHY, H. C., AND FRANCES MEEHAN. 1946. Reaction of oat varieties to a new species of Helminthosporium. Phytopathology **36**: 407.
15. PANTANELLI, E. 1921. Sui rapporti fra nutrizione e recettività per la ruggine. (The relation between nutrition and susceptibility to rust.) Riv. di Patol. Veg. **11**: 36-64. (Original not seen, abs. in Rev. Appl. Mycol. **1**: 118-121.)
16. PETURSON, B. 1930. Effect of temperature on host reactions to physiologic forms of Puccinia coronata avenae. Sci. Agr. **11**: 104-110.
17. SIMONS, M. D. 1954. The relationship of temperature and stage of growth to the crown rust reaction of certain varieties of oats. Phytopathology **44**: 221-223.

NUB-ROOT—THE EXPRESSION OF SEEDLING DISEASE IN THE MATURE COTTON AND FLAX PLANT [1]

Robert B. Stewart and Marvin D. Whitehead

SUMMARY

Nub-root is an apparently undescribed symptom of seedling disease of cotton, flax, and perhaps of other tap-rooted plants as expressed in the mature plant. Similar symptoms on cotton have heretofore been attributed to unfavorable soil structure. To test the hypothesis that the symptoms on the mature plant were incited by seedling-disease organisms, lesioned seedlings of cotton were selected and transplanted to sterile soil. Forty-six per cent of the lesioned seedlings developed nub-root while only 4 per cent developed nub-root in the apparently healthy seedlings used as controls. The usual seedling disease organisms, *Fusarium* spp., *Pythium* spp., and *Rhizoctonia solani* Kuhn, were isolated from the diseased seedlings.

When mycelium does not penetrate past the endodermal layer, the host may recover completely. The converse situation exists wherein the fungi ramify the tissue of the cortex and stele and kill the seedling (damping-off). Between these 2 extremes a situation exists which gives rise to root abnormalities.

These symptoms were observed to occur throughout the cotton-producing sections of Texas during 1952, 1953, and 1954 and were noted in Oklahoma in 1954. The percentage of affected plants varies considerably but some are nearly always present.

The consideration of nub-root as an effect of seedling disease in disease surveys will necessitate a considerable upward revision of yield losses attributed to the seedling disease complex.

Damping-off and sore-shin, seedling diseases incited by a complex of seed-borne and soil-borne organisms, are known to occur wherever cotton is grown.[2] They have been considered primarily as diseases of small

[1] Accepted for publication February 27, 1955.

[2] Neal, D. C., and W. W. Gilbert. 1935. Cotton diseases and methods of control. U.S. Dept. Agr. Far. Bul. 1745, 34 p.

seedlings and as occurring as a rot near the surface of the soil. Crop loss has been attributed to reduction of stand along with some aftereffect from stem cankers. Root malformation and deficiencies in the mature plant have been attributed to the effects of soil structure. Taubenhaus, Ezekial, and Rea[3] reported cotton roots to be constricted and the plant literally strangled to death by the compaction of heavy clay soil by rain and irrigation. Hubbard and Herbert[4] noted a similar reaction of cotton roots penetrating the coarse sand strata of soils at Shafter, California. Baily[5] described abnormal root development in the Egyptian Sudan and attributed it to soil hard pan. He observed varietal differences to the abnormal root development. In 1952, root abnormalities, here described as nub-root, were noted on plants growing in soils lacking an impervious layer. Later observations of root abnormalities in seedling cotton suggested the possibility that these symptoms may have resulted from damage during the seedling stage by one or more of the damping-off disease organisms.

SYMPTOMS.—The more severely affected cotton plants may wilt suddenly, or they may become stunted and generally unthrifty before eventually dying. Those less affected plants may show correspondingly less severe symptoms. Plants with moderate root abnormalities may show no aboveground symptoms. It is possible for wilt to occur when the soil-moisture content is high because the abbreviated root-system is unable to support the well developed shoot. In the more severe phase of nub-root, the tap root appears to have been cut off near the soil surface (Fig. 1, E, I, J, K). When the normal tap root system is destroyed, lateral roots may develop, permitting the plant to reach maturity (Fig. 1, B, C, G, H). Young lateral roots may become invaded and develop the same symptoms as the tap root (Fig. 1, B). When the root has been infected on 1 or both sides without having become completely girdled, an extended, flattened, blade-like root develops (Fig. 1, C, D, F, L). The same symptoms were noted to occur on roots of flax in a nursery at College Station, Texas (Fig. 2). In flax, however, the leaves on infected plants tend to be chlorotic and reddish (Fig. 2, B, D). Similar symptoms were noted on other tap-rooted plants but no investigations were made as to the inciting agents.

RESULTS AND DISCUSSION.—The cotton plant normally has a long slender tapering tap root (Fig. 1, A). Hubbard and Herbert[4] traced tap roots of cotton to a depth of 5 ft. When such a root system is compared with one which has typical nub-root, it is not difficult to understand the sudden wilting of such plants under water stress. The laterals, which develop from the injured roots, usually grow horizontally and the plants are able to thrive if moisture is available in the upper soil layers. They may often develop into what appear to be normal plants unless moisture becomes limiting. When moisture is limiting, they wilt and may die rather quickly.

In order to test the relationship between nub-root in the mature plant and seedling diseases incited by soil-borne organisms, seedlings were grown to a height of 3–4 in. in Lufkin fine sandy loam soil. The soil was washed from the seedlings and 75 showing root lesions were selected and transplanted to 25 1-gal. glazed crocks. An equal number of apparently healthy plants was used as a control. One seedling was removed from each pot at 1-month intervals and the root development examined.

Only 4 per cent of those plants grown from seedlings free of macroscopic lesions developed abnormal tap roots, while 46 per cent of those grown from seedlings with lesions developed nub-root symptoms (Table 1). This does not rule out the possibility of hard pan causing similar symptoms, but it does show that such symptoms can arise from invasions by pathogenic fungi.

Rhizoctonia solani generally is listed as the most severe incitant of seedling injury, but under conditions of Central Texas, *Fusarium* spp. are the most severe incitants. *Fusarium* spp., *Pythium* spp., and *Rhizoctonia solani* were isolated from the lesioned seedlings when plated on potato-dextrose agar. On further growth and maturation of the cotton plant, the lesions inflicted during seedling development healed over and no organism could be plated from the abnormal roots.

Microscopic study of diseased roots revealed that the fungal complex often did not penetrate past the endodermal layer. Under such conditions no lasting harm is done to the cotton plant, since the cortex is normally sloughed off by the maturing seedling. The converse situation exists wherein the fungi ramify throughout the tissue of the cortex and stele, inciting

[3] Science Service, Washington, D.C. Science News. Items. Science 71 (1847): XIV (Supplement).

[4] Hubbard, J. W., and F. W. Herbert. 1933. Root development of cotton in the San Joaquin Valley of California. U.S. Dept. of Agr. Circ. 262, 8 p.

[5] Bailey, M. A., and J. Templeton. 1926. A note on the abnormal behavior of cotton plants when subjected to handling. Egypt Mins. Agr. Tech. and Sci. Serv. Bul. 61, 8 p.

FIG. 1—2. Fig. 1. Symptoms of nub-root of cotton. A) Healthy root. B) Nub-root with secondary nubbing of lateral roots. C, D, F, K) Blade-like root development. G, H) Large lateral root development from nubbed plants. E, I, J) Severely nubbed-off root. L) Blade-like development of cotton root.—Fig. 2. Symptoms of nub-root of flax. A) Healthy plant. B) Lateral root development of infected plants showing proliferation. C, D) Plants with severely nubbed-off roots.

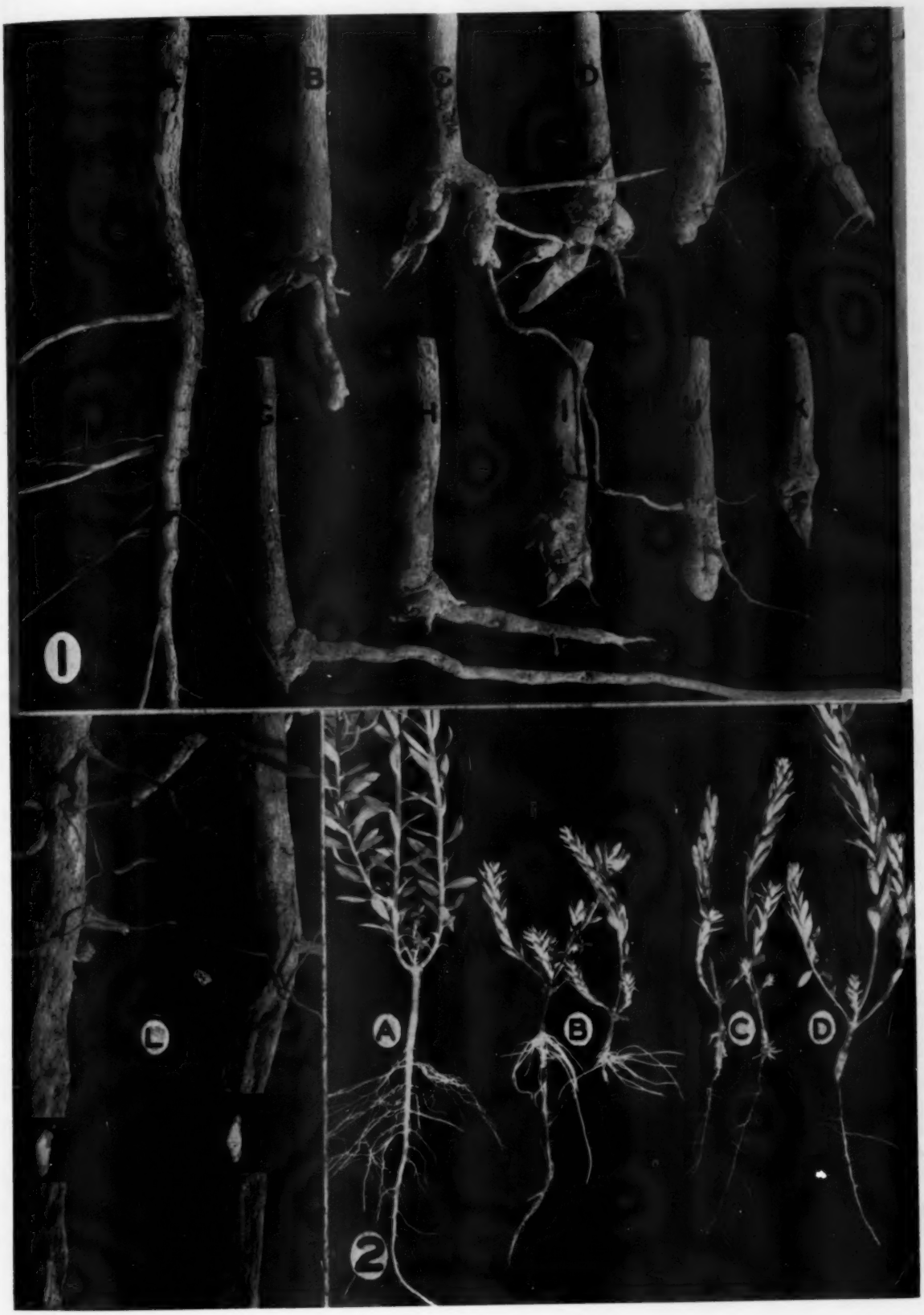
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TABLE 1.—*Incidence of nub-root in mature plants grown from apparently healthy seedlings and from lesioned seedlings*

Age of plant in months	Apparently healthy seedlings		Lesioned seedlings	
	Healthy plants	Nub-root	Healthy plants	Nub-root
1	25	0	11	14
2	24	1	17	8
3	23	2	12	13
Total	72	3	40	35

seedling death (damping-off). Between these 2 extremes a situation exists which gives rise to nub-root.

When the invading organism destroys the cambium in a given location within the root, and then for some reason (temperature, age of plant tissue, or other) fails to develop further, the root will form a callus and begin recovery. Because the cambium has been destroyed, this area of the root cannot develop further. Thus the degree and direction of the injury is determined by the extent of damage to the cambium tissue.

If the stele is invaded, the infection usually progresses until the root is severed. When this happens the growing point of the tap root has been removed and further downward growth of this organ is not possible. Lateral roots may then develop from the portion of the root above the lesion and the plant partly recovers from the disease. The remaining portion of the tap root may become somewhat enlarged because of an accumulation of carbohydrates that ordinarily would be utilized in development of a tap root.

If the organism does not completely sever the root of a seedling and destroys only a portion of it, a mature tap root conforming to the form of the remaining healthy tissue of the infected seedling root will develop. Thus, if the root is invaded on opposite sides, a flattened blade-like root will develop (Fig. 1, L). A histological study of roots of diseased seedlings showed that the form of the healthy or uninvaded tissue of an infected root conforms roughly to the root form attained in the maturing root.

To determine whether these symptoms were the result of simple tissue destruction or of a particular biological relationship, tap roots of cotton were altered mechanically in 3 ways: 1) roots were severed completely about ½ in. below soil line; 2) roots were split longitudinally and 1 half of root removed; and 3) roots were cut on 2 opposite sides, leaving a blade-like tap root. These plants were then transplanted to 6-in. clay pots and after 1 month they were removed and examined. Following these treatments, the plants developed tap roots which conformed to the resulting shape of the roots after portions of them were excised mechanically. The treatments in this experiment are thought to be analogous to the action of the organisms.

Seedling diseases of cotton (damping-off and sore-shin) often are not considered important by growers since it is common practice to plant more than enough seed and then to thin to a stand. This concept would be sound if recovery from seedling disease were complete. The symptoms here described show that recovery may not be complete.

TABLE 2.—*Distribution and prevalence of nub-root in 1953 and 1954*

Locations	Percentage nub-root	
	1953	1954
South Texas		
College Station		
Upland	47	42
Brazos River Bottom	25	90
Bryan	50	
Tabor	30	
Waco		95
Marlin		80
Calvert		30
North Texas[a]		
Ft. Worth		20
Lubbock		75
Idalou		51
Lorenzo		30
Ralls		14
So. Central Oklahoma		
Oklahoma City		30
Waurika		46
Chickasha		55

[a] The authors are indebted to Dr. Harry C. Lane, Texas Agricultural Experiment Station, Substation No. 8, Lubbock, Texas, for field readings made in North Texas.

In 1952 seedling diseases were estimated to cause 2.5 per cent loss in yield in Texas. Heretofore, nub-root has not been considered in seedling disease losses. In a number of fields examined in 1953 and 1954, a relatively high percentage of the plants were affected by nub-root (Table 2). During the past 3 years nub-root symptoms have been noted throughout the cotton-producing sections of Texas. One survey in South Central Oklahoma in August, 1954, revealed the presence of the disease in Oklahoma (Table 2). The counts do not take into account the loss of stand from earlier killing. Diseased plants that are not damped-off are considered part of the field stand and compete for plant nutrients along with healthy plants. They may die later when a water shortage develops, or they may continue to live and to produce poorly. The ultimate loss from this disease is conditioned by the prevalence of the disease, by the degree of host plant recovery, and by environmental factors. Apparently adequate moisture can do much to alleviate the loss due to the disease. It is considered that during the 1954 season the yield loss from seedling diseases, taking into account mature plant losses, will constitute the major cotton disease problem in Texas. During the 1954 season, yield loss in the fields surveyed was estimated at 5-75 per cent.

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ANTAGONISTIC AND STIMULATORY EFFECTS OF SOIL MICROORGANISMS UPON SCLEROTIUM ROLFSII [1]

D. J. Morton[2] and W. H. Stroube[3]

SUMMARY

Antagonistic effects of the soil microflora upon *Sclerotium rolfsii* Sacc. were studied with microorganisms isolated at random from soils and tested individually in agar cultures. Two of 1114 bacteria (0.2 per cent), 18 of 1064 actinomycetes (1.7 per cent), and 35 of 1005 fungi (3.5 per cent) inhibited growth of *S. rolfsii*. In greenhouse tests with the antagonistic organisms and *S. rolfsii*, the fungi, the actinomycetes, and the bacteria significantly reduced disease severity.

It has been reported that *S. rolfsii* will not grow well on synthetic media unless thiamin is added, and also that many microorganisms produce this vitamin. In this study, *S. rolfsii* grew well on a medium lacking thiamin (Czapek's sucrose-nitrate agar) in the presence of certain other microorganisms, suggesting that an essential metabolite, such as thiamin, was being provided by the added organisms. Tests revealed that 842 of 1036 bacteria (81.3 per cent), 660 of 1005 actinomycetes (65.7 per cent), and 826 of 1219 fungi (67.7 per cent) stimulated *S. solfsii* in this manner. In greenhouse tests, no significant increases in disease severity occurred when microorganisms stimulatory to *S. rolfsii* in agar culture were used, indicating that the soil microflora does not have a stimulatory effect upon the fungus under natural conditions.

Southern blight, caused by *Sclerotium rolfsii* Sacc., is a serious disease of many crops in the southern areas of the United States. An outstanding feature of this disease on soybeans is its erratic nature. It often attacks isolated plants in a field, leaving small areas of diseased plants scattered throughout otherwise healthy stands. Atkins (2) suggested that temporary variations in the numbers of soil microorganisms might account for this phenomenon, hence, a study of antagonistic and stimulatory effects which the major groups of soil microorganisms have upon *S. rolfsii* was initiated.

Antagonistic effects were given greater emphasis than stimulatory ones, since much evidence of the former has been reported with other soil pathogens (2, 4, 15). Considering the importance of *S. rolfsii* as a plant pathogen, relatively little work has been done on influence of soil microflora upon this fungus. McClellan et al (9) suggested that a reduction in germinability of sclerotia, which they observed, was due to action of antagonistic organisms, and Johnson (7) demonstrated that soil microflora may be antagonistic to *S. rolfsii*. Atkins (2) reported that a bacterium reduced pathogenicity of *S. rolfsii* to soybean and to Crotalaria seedlings in greenhouse tests. Porter (11) found that *S. rolfsii* was inhibited by *Bacillus subtilis* (Cohn) Prazmowski, and Weindling (15) noted that the fungus was more or less susceptible to attacks of *Trichoderma lignorum*. Hine and Endo (5) stated that *T. viride* Fries was able to attack and destroy sclerotia and mycelium of *S. rolfsii*.

Stimulatory effects also were considered in this study. Examples of such relationships among microorganisms include Vuillemin's description (14) of the beneficial effects of *Mucor rouianus* (Calmette) Wehmer upon a micrococcus, Molliard's reference (10) to members of the genus *Ascobolus* which produced perithecia only in the presence of bacteria, and Zeller and Schmitz's report (16) of fungal growth and sporulation stimulated by other fungi. A possible method of investigating stimulatory effects upon *S. rolfsii* was suggested by the works of Joham (6) and Thompson (13). Joham showed that thiamin chloride was essential for normal growth of *S. rolfsii*, and Thompson reported that several common species of bacteria actively produce thiamin in relatively large quantities.

MATERIALS AND METHODS.—Effects of soil microflora upon *S. rolfsii* were studied by isolating soil microorganisms, testing them for antagonistic and stimulatory effects in the laboratory, and finally testing them for such effects in greenhouse experiments. An isolate of *S. rolfsii* from an infected soybean plant was used throughout the study. Isolations of microorganisms from soil were made from 3 experimental farms near Baton Rouge, Louisiana, by means of standard techniques and the following media and dilutions: For bacteria, soil-extract agar (1) and a soil:water dilution of $1{:}10^8$; for actinomycetes, Conn's glycerol-asparaginate agar (8) and a soil:water dilution of $1{:}10^5$; and for fungi, rose bengal agar (12) and a soil:water dilution of $1{:}2 \times 10^4$. The plates were incubated at 28°C for 4 days with fungi, 6 days with actinomycetes, and 8 days with bacteria. Isolates were then selected at random and screened for antagonism to *S. rolfsii* on potato-dextrose agar by streaking 4 to a plate as described by Cooper and Chilton (3). Those

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isolates which were not overgrown by *S. rolfsii* in the screening tests were retested individually for antagonism. In the case of bacteria and actinomycetes, inhibition zones were measured after intervals of 1 and 2 weeks whereas with fungal isolates, records were made of whether or not the isolates overgrew *S. rolfsii*. All tests for antagonism were carried out at 28°C. Some examples of antagonistic actinomycete isolates are shown in Figure 1.

In tests on possible stimulation of *S. rolfsii* by soil microorganisms, the fungus was grown on Czapek's sucrose-nitrate agar (8), a medium which does not support much growth of *S. rolfsii* because it does not contain thiamin. Two small pieces of potato-dextrose agar infested with *S. rolfsii* were placed on opposite sides of each petri dish of Czapek's medium. A different soil isolate was then streaked next to each piece of infested agar. Control plates were similar, except

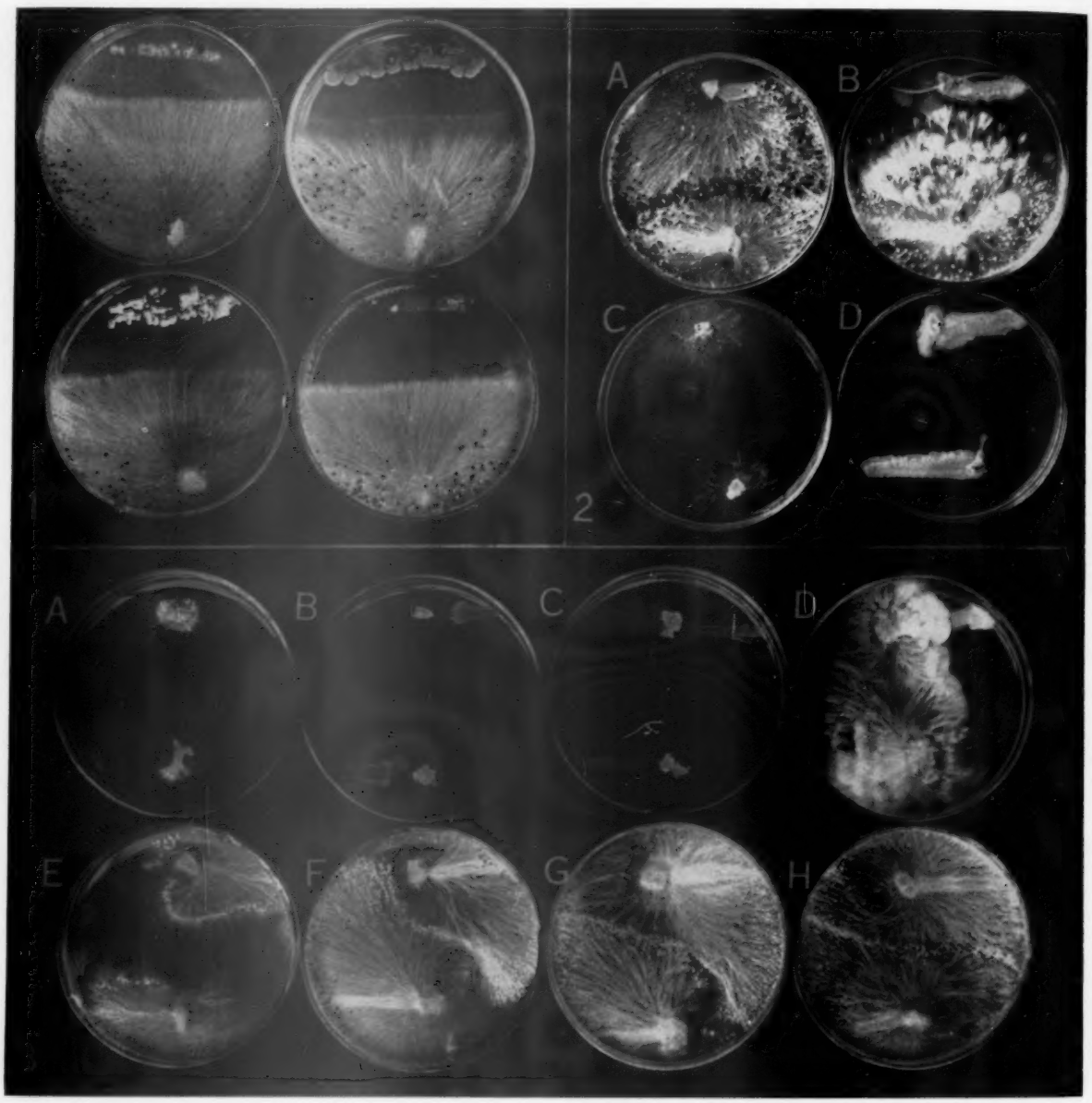

FIG. 1–3.—FIG. 1. Four actinomycete isolates antagonistic to *S. rolfsii* on potato-dextrose agar. The inhibition zones of 14-day-old cultures are shown.—FIG. 2. Stimulatory effects of bacterial isolates from soil upon *S. rolfsii* on Czapek's sucrose-nitrate agar: A) Both bacteria have stimulatory effect; B) One bacterium has a stimulatory effect; C) Control, no bacteria added; D) Neither bacterium has a stimulatory effect.—FIG. 3. Differences in stimulatory effects of 7 species of bacteria upon *S. rolfsii* on Czapek's sucrose-nitrate agar: A) Control (no bacterium added); B) *Pseudomonas synxantha;* C) *Aerobacter cloacae;* D) *Aerobacter aerogenes;* E) *Serratia marcescens;* F) *Bacillus alvei;* G) *Pseudomonas mucidolnas;* H) *Pseudomonas syncyanea.*

that no soil isolates were streaked on the plates. The plates were incubated for 4 days. If growth of *S. rolfsii* in the plates streaked with soil isolates was considerably greater than that in the controls, the isolate was considered to have a positive stimulatory effect (Fig. 2).

Many of the soil isolates which had antagonistic or stimulatory effects upon *S. rolfsii* in agar cultures were tested further in the greenhouse. The test plant was the Ogden variety of soybean. Seeds were planted in 4-in. pots of sterilized sandy loam, 10 seeds per pot. Disease severity was determined by counting the healthy seedlings 2 weeks after planting. All experiments were conducted in quadruplicate.

Three methods of soil infestation were used. In the first of these, cultures of *S. rolfsii* and the isolate being tested were grown for 1 week on potato-dextrose agar, after which the agar was cut into small blocks and ½ of a petri-dish culture of each organism was thoroughly mixed with the upper 2 in. of soil in the pots. With the second method, the liquid potato-dextrose medium was used and the contents of 1 petri dish containing *S. rolfsii* and those of 1 containing the soil organism were poured over the soil and covered in turn with more soil. Where mycelial mats were formed on the surface of liquid cultures, the mats were cut into small pieces and added to the soil.

In the third method, a cornmeal-sand medium was prepared by mixing 1000 g of sand, 200 g of cornmeal and 250 ml of water. The mixture was placed in 500-ml flasks, sterilized, and seeded with the test organism. After a week's incubation, during which each flask was shaken daily, the mixture was added to soil in the pots at a rate of from 2 to 5 per cent by volume for each organism. Two series of controls were used in all of the experiments. In 1 series, soybean seeds were planted together with 1 portion of sterile medium and 1 of medium infested with *S. rolfsii;* in the other, soybean seeds were planted in the presence of 2 portions of sterile medium.

RESULTS.—The group of soil organisms having the highest proportion of isolates antagonistic to *S. rolfsii* was the fungi (35 of 1005 tested or 3.5 per cent). A lower proportion (18 of 1064 or 1.7 per cent) of actinomycete isolates had this effect, and only 2 of 1114 bacterial isolates (0.2 per cent) were antagonistic. The 2 antagonistic bacteria were identified as strains of *Bacillus subtilis,* the 35 fungi as *Trichoderma* spp., and the 18 actinomycetes as species of *Streptomyces.* The antagonistic bacteria and actinomycetes produced an inhibition zone, whereas the fungi overgrew *S. rolfsii.* In general, the actinomycetes formed wider zones than did the bacteria.

In preliminary experiments dealing with effects of antagonistic microorganisms upon *S. rolfsii* in soil, 3 methods of soil infestation were used: the agar-plate method, the liquid-culture method, and the cornmeal-sand method. In further experiments the agar-plate method was abandoned because pathogenic effects produced were too mild, and results obtained with it were not statistically significant.

It may be noted that more of the isolates gave significant control when the cornmeal-sand method was used than when the liquid-culture method was used (Table 1). In all 4 tests, the 2 fungal isolates and actinomycete isolates 6, 9, and 11 were significantly effective in controlling the disease. Actinomycete isolates 3, 5, 7, 8, and 10 were each effective in 2 tests, whereas actinomycete isolate 1 and both bacterial isolates each gave significant control in 1 test. No correlation was found between the width of the inhibition zones of the different isolates in agar-plate cultures and the degree of disease control produced by the same isolates.

A greater proportion of the bacteria (842 of 1036 tested or 81.3 per cent) had a stimulatory effect upon *S. rolfsii* on Czapek's medium than was the case with the actinomycetes or the fungi. The proportion of actinomycetes and fungi that produced stimulatory effects was 660 of 1005 (65.7 per cent) and 826 of 1219 (67.7 per cent), respectively.

Thirteen species of bacteria also were tested for stimulatory effects. Those which enabled *S. rolfsii* to grow vigorously were *Pseudomonas mucidolans* Levine & Anderson, *Pseudomonas syncyanea* (Ehrenberg) Migula, *Bacillus alvei* Cheshire & Cheyne, *Aerobacter aerogenes* (Kruse) Beijerinck, and *Serratia marsescens* Bizio; those which stimulated growth moderately were

TABLE 1.—*The number of healthy soybean seedlings grown from seed planted in soil infested with S. rolfsii and with antagonistic organisms*

Isolate	Method of infesting soil			
	Liquid culture		Cornmeal-sand culture	
	Test 1[a]	Test 2	Test 1	Test 2
Bacterium No. 1	7.50[b]	5.75	1.50	3.50**
Bacterium No. 2	7.00	5.75	0.75	2.50**
Actinomycete No. 1	8.50**[c]	6.00	0.25	0.75
Actinomycete No. 2	6.50	5.00	0.25	0.00
Actinomycete No. 3	8.50**	6.75	5.00**	1.25
Actinomycete No. 4	8.00**	8.75**	6.75**	6.75**
Actinomycete No. 5	5.50	9.00**	5.75**	1.50
Actinomycete No. 6	7.75**	8.75**	6.25**	9.00**
Actinomycete No. 7	7.25	7.00	6.50**	8.25**
Actinomycete No. 8	6.50	7.50	8.50**	9.25**
Actinomycete No. 9	8.00**	7.75**	8.00**	9.00**
Actinomycete No. 10	7.00	7.50	4.25**	4.25**
Actinomycete No. 11	9.25**	9.50**	7.75**	7.00**
Fungus No. 1	8.00**	9.75**	7.75**	10.00**
Fungus No. 2	8.75**	9.00**	7.75**	9.50**
Control (infested)	5.75	5.25	0.00	0.00
Control (non-infested)	9.00**	9.75**	8.50**	9.75**
LSD (.01)	1.98	2.47	2.24	2.28

[a] Each test is the average of 4 replications, with 10 seeds used in each.

[b] Average number of healthy seedlings produced from 10 seeds.

[c] **Significant decrease in pathogenicity from infested control at 1 per cent level.

Salmonella anatis (Rettger & Scoville) Bergey et al, *S. pullorum* (Rettger) Bergey et al, *Escherichia coli* (Migula) Castellani & Chalmers, *E. freundii* (Braak) Yale, *Lactobacillus casei* (Orla-Jensen) Holland, and *Bacillus pumulus* Gottheil; whereas those which did not favor growth of the fungus were *Pseudomonas synxantha* (Ehrenberg) Holland and *Aerobacter cloacae* (Jordon) Bergey et al. Some examples of these results are shown in Figure 3.

In all cases in which a microorganism did not stimulate the growth of *S. rolfsii*, the growth of the fungus was completely inhibited. In the plates containing only *S. rolfsii*, however, a small amount of growth occurred. This suggests that the microorganisms which were not stimulatory produced some substance that inhibited the growth of *S. rolfsii* on Czapek's medium. Such a substance probably would not have to be very potent as the medium itself is unfavorable to the growth of *S. rolfsii*.

In order to determine whether or not thiamin would produce an effect upon *S. rolfsii* similar to that of the stimulatory microorganisms, this vitamin was added as thiamin hydrochloride to Czapek's medium in 8 concentrations ranging from 10^{-3} to 10^{-10}. In all concentrations of 10^{-8} or greater, *S. rolfsii* grew as vigorously as it did when stimulatory microorganisms were present, whereas in all concentrations lower than this, growth of the fungus was poor.

Greenhouse experiments were designed to determine whether organisms that produced stimulatory effects in the laboratory might increase pathogenicity of *S. rolfsii* in greenhouse culture. Six species of bacteria were chosen for this test. In agar-plate tests, 2 (*P. mucidolans* and *A. aerogenes*) were highly stimulatory, 2 (*E. coli* and *B. pumulus*) were moderately stimulatory, and 2 (*P. synxantha* and *A. cloacae*) had no stimulatory effects. The liquid-culture method of infestation was chosen in preference to the others, since the degree of pathogenicity obtained with infested controls using this method was only moderate, and consequently any increase in disease severity could be measured. The experiment was conducted twice in quadruplicate, and in only 1 case was a bacterium associated with a significant increase in pathogenicity of *S. rolfsii*. This organism, *A. cloacae*, was not stimulatory in agar-plate tests and no increase in disease severity occurred when this bacterium was retested in the soil.

DISCUSSION.—In the present studies, it was found that only a small proportion of the soil microrganisms tested were antagonistic to *S. rolfsii*. From the results obtained, it seems probable that fungi play the most significant role in any antagonism that soil microflora may have towards *S. rolfsii*. A few of the actinomycetes might also be important, whereas the bacteria would seem to be of little importance.

In the experiments concerned with stimulatory effects of microorganisms upon *S. rolfsii*, it was found that a relatively high percentage of the soil organisms apparently produced thiamin, since the fungus grew well in their presence on a thiamin-deficient medium. Organisms that were able to stimulate growth of *S. rolfsii* on thiamin-deficient medium failed to increase consistently the pathogenicity of the fungus to soybean plants in greenhouse tests. It appears unlikely, therefore, that the natural soil microflora has a stimulatory effect upon *S. rolfsii* by producing thiamin. The fact that *S. rolfsii* grows well on soil-extract agar (1), a medium containing soil filtrate as the only source of organic material, indicates that soil contains amounts of thiamin in excess of the minimum requirements of the fungus so that any such vitamin produced by soil microorganisms would not be needed by the fungus.

LOUISIANA AGRICULTURAL EXPERIMENT STATION
BATON ROUGE, LOUISIANA

LITERATURE CITED

1. ALLEN, O. N. 1951. Experiments in soil bacteriology. Burgess, Minneapolis. 126 p.
2. ATKINS, J. G. 1951. Annual Progress Report. Dept. Pl. Path., La. Agr. Exp. Sta. (Unpublished)
3. COOPER, W. E., AND S. J. P. CHILTON. 1950. Studies on antibiotic soil organisms. I. Actinomycetes antibiotic to Pythium arrhenomanes in sugar-cane soils of Louisiana. Phytopathology **40**: 544-552.
4. GARRETT, S. D. 1940. Soil conditions and the take-all disease of wheat. V. Further experiments on the survival of Ophiobolus graminis in infected wheat stubble buried in the soil. Ann. Appl. Biol. **27**: 199-204.
5. HINE, I., AND E. ENDO. 1940. Trichoderma parasitic on sclerotial fungi. Japan. Ann. Phytopath. Soc. **10**: 31-241.
6. JOHAM, H. E. 1943. A nutritional study of the fungus Sclerotium rolfsii. M.S. Thesis. Agr. and Mech. Col. of Texas.
7. JOHNSON, S. P. 1953. Some factors in the control of the southern blight organism, Sclerotium rolfsii. Phytopathology **43**: 363-368.
8. LEVINE, M., AND H. W. SCHOENLEIN. 1930. A compilation of culture media for the cultivation of microorganisms. Williams and Wilkins, Baltimore.
9. MCCLELLAN, W. D., J. R. CHRISTIE, AND N. L. HORN. 1949. Efficacy of soil fumigants as affected by soil temperature and moisture. Phytopathology **39**: 272-283.
10. MOLLIARD, M. 1903. Rôle des bacteries dans la production des perithèces des Ascobolus. [Paris] Acad. des Sci. Compt. Rend. **136**: 899-901.
11. PORTER, C. M. 1924. Concerning the characters of certain fungi as exhibited by their growth in the presence of other fungi. Amer. Jour. Bot. **11**: 168-188.
12. SMITH, N. R., AND V. T. DAWSON. 1944. The bacteriostatic action of rose bengal in media used for plate counts of soil fungi. Soil Sci. **58**: 467-471.
13. THOMPSON, R. C. 1942. Synthesis of B vitamins by bacteria in pure culture. Univ. of Texas Publ. 4237: 87-96.
14. VUILLEMIN, P. 1902. Sur les effets du commensalisme d'un Amylomyces et d'un Micrococcus. [Paris] Acad. des Sci. Compt. Rend. **134**: 366-368.
15. WEINDLING, R. 1932. Studies on a lethal principle effective in the parasitic action of Trichoderma lignorum on Rhizoctonia solani and other soil fungi. Phytopathology **24**: 1153-1179.
16. ZELLER, S. M., AND H. SCHMITZ. 1919. Studies in the physiology of the fungi. VIII. Mixed cultures. Mo. Bot. Gard. Ann. **6**: 183-192.

A BROADER CONCEPT OF THE SPECIES SCLEROTINIA SCLEROTIORUM BASED ON VARIABILITY [1]

Laurence H. Purdy[2]

SUMMARY

Single ascospore cultures were obtained through 2–3 generations from isolates tentatively identified as *S. sclerotiorum*, *S. trifoliorum*, or *S. minor* based on host association and sclerotial size. Measurements of asci and ascospores from each generation were recorded and compared with those given in the original descriptions. It was found that there was no line of demarcation between the ascus or ascospore measurements of the species studied, and it was impractical, if not impossible, to distinguish the asci or ascospores of 1 species from those of the other.

Cultural variants were obtained varying from those that produced no sclerotia to those that produced very large sclerotium-like masses. A continuous intergradation in sclerotium size was obtained from small sclerotia of *S. minor* or *S. intermedia* to the large ones of *S. trifoliorum* and *S. sclerotiorum*. Species could not, therefore, be separated with certainty on the basis of sclerotium size.

The species referred to in the literature as *S. minor*, *S. trifoliorum*, *S. trifoliorum* var. *fabae*, *S. intermedia*, *S. sativa*, and *S. sclerotiorum* comprise a natural group which produces apothecia and true sclerotia, lacks conidia, and attacks a wide range of host plants. Since it is impossible to identify these species in practice because of the variability of the taxonomic characters that have been used, it is proposed that the name *S. sclerotiorum* be applied to large- and small-sclerotium types regardless of the host involved.

It is further proposed that where it is of some practical convenience to do so, the former species such as *S. intermedia* and *S. minor* be considered as "horticultural varieties" of the species *S. sclerotiorum* and be referred to, for example, as *S. sclerotiorum* "Intermedia" and *S. sclerotiorum* "Minor", respectively, and that the large-sclerotium forms be referred to as *S. sclerotiorum* "Major".

The present system of species separation of several of the plant pathogenic members of the genus *Sclerotinia* emphasies size of sclerotia and host association. Individual isolates that differ in cultural characteristics or host association from isolates identified as *Sclerotinia sclerotiorum* (Lib.) D By. have been described as new species. For example, large-sclerotium isolates obtained from forage legumes usually have been placed in the species *Sclerotinia trifoliorum* Eriks., while similar or identical appearing isolates obtained from nonlegumes usually have been designated as *S. sclerotiorum*. Inconsistent with this system, however, is the designation of the Sclerotinia on snap beans as *S. sclerotiorum* merely because the host is a vegetable legume rather than a forage legume. A further inconsistency is found in the designation of small-sclerotium isolates from a wide variety of hosts as *Sclerotinia minor* Jagger while reserving the name *Sclerotinia sativa* Drayt. & Groves for those isolates obtained from some clovers and bulbous plants. Erection of these species purportedly has been based on slight differences in the perfect stage. However, vegetative or reproductive variants have been described as new species. The practice of erecting species based on 1 or a few isolates has not taken into consideration the range of variation of taxonomic characters, for if it had these species would not have been erected.

It became apparent that the taxonomy of these species of *Sclerotinia* is not satisfactory, and the present study was initiated from the standpoint of a modern philosophy of speciation based on a knowledge of variation (23, 24).

REVIEW OF LITERATURE.—*Peziza sclerotiorum* was originally described by Libert, but this binomial was changed by de Bary (8) to *S. sclerotiorum* in 1884. According to Saccardo (22), Libert gave ascus and ascospore measurements of 130–135 × 8–10 μ and 9–13 × 4–6.5, respectively. A very liberal interpretation of this description has been taken. For example, Ramsey (21) studied 28 isolates that were identified as *S. sclerotiorum* primarily on sclerotium size, but the ascus and ascospore measurements ranged far beyond the limits set forth in the original description. A similar interpretation was made by Dickson (9) who studied 33 isolates identified as *S. sclerotiorum*. Dickson found that the character of growth varied from appressed to aerial and that differences in growth rates occurred among the different isolates. He stated that if several of the isolates had been studied separately, they could have been described as new species. However, when studied as a group an intergradation from one type to another was observed. Data from the literature (Table 1) show that measurements of asci and ascospores are quite variable for different isolates identified by several different workers as *S. sclerotiorum*. These data also show that the asci have been reported to vary from 81.0 to 199.4 μ in length and 4.3 to 12.4 μ in width and the ascospores

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from 6.0 to 17.0 μ in length by 2.0 to 8.4 μ in width. It is likely that the original description of this species by Libert was based on very few measurements and perhaps even on a single specimen.

The measurements given by Saccardo (22) for *S. trifoliorum* (Table 1) are not in agreement with those presented by Ericksson (11) in the original description of the species. Ericksson gave 160.0 μ as the ascus length, Saccardo 180.0 μ. These figures are probably averages because no variation was reported by either author. The asci of *S. trifoliorum* have been reported to vary from 140.0 to 234.0 μ in length by 8.0 to 22.4 μ in width and the ascospores from 8.3 to 19.9 μ in length by 5.0 to 10.0 μ in width. In the variety *fabae* some measurements were still larger. Identification of isolates of *Sclerotinia* as *S. trifoliorum* is often based on association with forage legumes (12, 18). Such a basis of identification is not reliable however, since the host ranges of *S. trifoliorum*, *S. sclerotiorum*, and *S. minor* overlap (3, 12, 13, 14, 16, 25). Kreitlow (18) reported 4 cultural types of *S. trifoliorum* that originated from single ascospores. These ranged from nonsclerotial types to those that produced abundant sclerotia and also differed in rate of growth.

S. minor was described by Jagger (14) as a distinct species that differed from *S. sclerotiorum* in having smaller sclerotia. The ascus and ascospore measurements reported by several workers (Table 1) for this species are well within the range of variation reported for *S. sclerotiorum* (9, 21). Chivers (5) reported the sclerotium size as 0.25–5.00 mm. He showed that temperature affected the size of sclerotia, the largest occurring at 25°C, and that sclerotium size also depended on the isolate. Balfe (1) reported a nonsclerotium type obtained from sclerotial transfers of *S. minor*. This type was apparently similar to that reported for *S. trifoliorum* by Kreitlow (18). Balfe (1) also reported a buff colored mycelial type from *S. minor*.

Ramsey (20) described *S. intermedia* as a new species with sclerotia intermediate in size between those of *S. sclerotiorum* and *S. minor*, and reported differences in ascus and ascospore size. He placed much emphasis on the fact that this species grew faster at low temperatures than did either *S. minor* or *S. sclerotiorum*. The average ascus and ascospore measurements which he reported for *S. intermedia* (127.0 × 7.5 μ and 12.7 × 4.9 μ, respectively) agree very closely with those reported by Ramsey (21) and Dickson (9) for *S. sclerotiorum*. Chivers (5) showed that the sclerotia of *S. intermedia* vary with the temperature from 0.75–6.0 mm in diameter.

Drayton and Groves (10) described a small-sclerotium type associated with bulbous plants and clovers as a new species, *S. sativa*, and gave ascus and ascospore measurements of 100.0–130.0 × 7.5–10.0 μ and 9.0–12.0 × 4.0–7.0 μ, respectively. Sclerotia were reported as 1–5 mm long and 1–2 mm thick, dimensions within the range reported for *S. minor* (14), *S. intermedia* (20), and *S. sclerotiorum* (9, 21). A culture of *S. sativa* furnished by Constance A. Loveland, Science Service, Division of Botany and Plant Pathology, Ottawa, Canada, was indistinguishable culturally from other small-sclerotium isolates studied herein.

Keay (15) obtained ascus measurements from *S. sclerotiorum*, *S. trifoliorum*, *S. trifoliorum* var. *fabae*, and *S. minor* and on the application of Fisher's t-test reported that "the means (for the asci of different described species) were all found to differ significantly, but this was also true when samples of the same fungus were compared". This indicated that the range of variation of ascus measurements within a species may be as great or greater than the variation of measure-

TABLE 1.—*Measurements reported in the literature for the asci, ascospores, and sclerotia of several species of the genus Sclerotinia*

Species	Author	Asci (μ)	Ascospores (μ)	Sclerotia (mm)
S. sclerotiorum	Libert (22)	130.0–135.0 × 8.0–10.0	9.0–13.0 × 4.0–6.5	30
	Ramsey (21)	125.4–160.4 × 8.2–10.2	11.7–15.1 × 5.9–7.3	2.5–6
	Dickson (9)	124.4–147.5 × 8.1–10.2	9.5–13.5 × 4.8–6.8	2–3 × 6–7
	Davis (7)	100.0–125.0 × 5.0–9.0	9.0–11.0 × 4.0–6.0	1–30
	Bisby (3)	100.0–150.0 × 8.0–10.0	10.0–14.0 × 5.0–6.5	
	Parievskaya (19)	152.9–199.4 × 4.3–12.4	9.8–16.8 × 4.2–8.4	
	Kheswalla (16)	81.0–127.0 × 6.8–10.2	7.4–15.3 × 4.4–6.8	4–15
	Young & Morris (27)	83.0–140.0 × 6.0–11.0	6.0–17.0 × 2.0–8.0	2–10
S. trifoliorum	Erickson (11)	160.0 × 10.0	15.0–17.0 × 8.0–10.0	
	Saccardo (22)	180.0 × 12.0	14.0–18.0 × 6.0–8.0	
	Wolf & Cromwell (26)	140.0–160.0 × 8.0–11.0	12.0–14.0 × 8.0	0.3–10
	Keay (15)	147.0–234.0 × 9.3–19.6	8.3–19.9 × 5.0–8.8	
var. *fabae*	Keay (15)	165.0–252.2 × 10.0–22.4	10.0–28.2 × 6.4–15.2	
	Jagger (14)	125.0–175.0 × 8.0–11.0	8.3–19.9 × 5.0–8.8	0.5–2
	Keay (15)	128.0–182.0 × 8.0–11.0	10.0–19.0 × 5.5–10.0	
S. minor	Ramsey (21)	141.0 × 8.9	14.1 × 7.0	0.5–1
	Beach (2)	150.0–165.0 × ?	11.0–18.0 × 6.0–8.0	
	Chivers (5)			0.25–5
S. intermedia	Ramsey (20)	121.6–131.4 × 7.2–7.7	10.4–15.2 × 3.6–5.7	1–3
	Chivers (5)			0.75–6
S. sativa	Drayton & Groves (10)	100.0–130.0 × 7.5–10.0	9.0–12.0 × 4.0–7.0	1–5 × 1–2

ments of different described species. She also reported that ascospore length measurements of the species studied were significantly different, but significant differences were also found among the ascospore lengths of different isolates of the same species.

With regard to host association, Dickson (9) has shown that the host range of *S. sclerotiorum* is very wide, whereas Frandsen (12) has listed only a relatively few hosts for *S. trifoliorum*. This is undoubtedly due to the designation of large-sclerotium isolates from nonforage legumes as *S. sclerotiorum* while only large-sclerotium types from forage legumes are identified as *S. trifoliorum*. Small-sclerotium isolates from forage legumes have been identified as *S. trifoliorum* or *S. sativa*, while those from nonforage legumes have been identified as *S. minor*.

The similarities in the species *S. trifoliorum* and *S. minor* led Valleau, Fergus, and Henson (25) to the conclusion that they may be synonymous, since no real difference between the 2 was observed. Cunningham (6) stated that the description of *S. intermedia* and *S. minor* agree with the description of *S. sclerotiorum*, and that slight differences in certain characters "cannot be considered as possessing specific value."

MATERIALS AND METHODS.—All isolates of *S. sclerotiorum* and *S. minor* and almost all isolates of *S. trifoliorum* were obtained from infected host tissue. Sclerotia or tissue pieces containing mycelium were surface sterilized in 1:10 sodium hypochlorite solution for 10 minutes prior to placing them on potato-dextrose agar plates. Pure cultures were obtained by transferring hyphal tips to potato-dextrose agar slants.

Single-ascospore cultures were obtained from apothecia by placing an apothecium at the edge of a water-agar plate so that the hymenial surface was perpendicular to the agar surface and facing the center of the plate. The plate was tilted about 10° from the vertical so that ascospores alighted on the agar surface as they were forcibly ejected. Ascospores that were sufficiently isolated from one another could be picked up without difficulty under the medium power (30×) of a dissecting microscope.

To induce apothecium production in the laboratory, sclerotia from potato-dextrose agar slants or plates about 1 month old were transferred to sterile water blanks containing about 15 ml of water. The tubes were shaken so that the entire surface of each sclerotium was wetted and mycelium adhering to the surface removed so that a continuous water film was present. The sclerotia and wash water were poured into sterile petri plates and placed at 15°C until apothecial initials appeared, at which time the plates were placed beneath a bank of six 100-watt Mazda lamps in a room held at 12°C. Illumination was provided 12 hours each day.

Twenty to 40 single-ascospore cultures were obtained from each apothecium and were maintained on potato-dextrose agar slants at room temperature (18–25°C). Daily observations were made to note differences in growth rates and any other variations. Of the 20 or more single-ascospore cultures from each apothecium usually only 5 were kept for further study.

Thirty asci and 30 ascospores from each of 3 different apothecia obtained from each isolate were measured. The average and range of variation were recorded as though the total of 90 measurements were from a single apothecium. Only those asci containing 8 well-defined ascospores were measured, and all ascospore measurements were obtained from ascospores floating free in the mounting medium. On the basis of these measurements, cultural characteristics, and host association, the isolates were tentatively identified, when possible, as one of the presently recognized species of the genus.

TENTATIVE IDENTIFICATION OF ISOLATES.—*S. sclerotiorum*.—Isolates were tentatively identified as *S. sclerotiorum* on the basis of sclerotium size and host association. Almost all isolates so identified were of the large-sclerotium type (3–10 × 3–7 mm), but ascus and ascospore measurements were similar to those given for *S. sclerotiorum* (9, 21), *S. intermedia* (20), and *S. minor* (14).

One isolate from diseased lettuce differed considerably from all others. Sclerotia were large, irregular, and of a punky consistency not previously described. Abundant aerial brown mycelium was produced. Apothecia obtained from this isolate were whitish-cream in color with an irregularly shaped disc and a short stipe. On the basis of ascus and ascospore measurements, however, it was considered to be a variant of *S. sclerotiorum*.

All isolates tentatively classified as *S. sclerotiorum* were placed in 3 cultural types on the basis of sclerotium size and characteristics of mycelium on potato-dextrose agar at 18°C. A description of each type is as follows: *Type 1.* Sclerotia few, 3–10 × 3–7 mm, lenticular, black and hard at maturity with complete outer covering, interior white. Mycelium white and appressed to agar surface. *Type 2.* Sclerotia many, 2–7 × 2–4 mm, usually spherical, black and hard at maturity with complete outer covering, interior white. Mycelium white and aerial, not appressed to agar surface. *Type 3.* Sclerotia few, 4–25 × 3–11 mm, irregular to elliptical and flattened, incomplete black outer covering exposing pink interior, punky and soft. Mycelium brown, not appressed to agar surface, tufted, and aerial.

S. trifoliorum.—Since identification of *S. trifoliorum* usually has been based on its large sclerotia and on its pathogenicity to forage legumes, this was the basis adopted for identification of isolates studied herein. Several isolates from forage legumes were obtained from L. Henson and K. W. Kreitlow. All isolates studied were similar in cultural characteristics with only slight variation in sclerotial size and amount of surface mycelium.

The isolates tentatively identified as *S. trifoliorum* may be characterized as follows: Sclerotia few to many, 2–10 × 2–5 mm, black and hard at maturity, interior white, mycelium white, appressed to agar surface.

S. minor.—Identification of *S. minor* was based mainly on sclerotium size. Isolates with small sclerotia were tentatively identified as *S. minor* regardless of the host, and were characterized as follows: Sclerotia many, 0.5–4.0 ×0.5–2.0 mm, black and hard at maturity, outer covering complete, interior white. Mycelium white, appressed to agar surface.

S. intermedia.—On the basis of sclerotium size, several isolates were tentatively identified as *S. intermedia*. These were later tentatively identified as *S. sclerotiorum* or *S. minor*, however, based on a comparison of their ascus and ascospore measurements and sclerotial size with those given for *S. sclerotiorum* (9, 21), *S. intermedia* (20), and *S. minor* (14). There have been very few reports concerning *S. intermedia* since it was described by Ramsey (20). This indicates that either the species is extremely rare, or that it has been identified by other workers as some other species.

S. sativa.—An isolate of *S. sativa* was compared with isolates tentatively identified as *S. minor* and was found to be indistinguishable from *S. minor* as to sclerotium size and cultural characteristics. Apothecia of *S. sativa* were not obtained, however, and ascus and ascospore measurements could not be compared with those of the other isolates studied. Since this species has been reported only once, and ascus and ascospore measurements (10) were within the range reported for *S. sclerotiorum* (9, 21), it is probable that it is a variant of *S. sclerotiorum* rather than a distinct species.

Ascus and ascospore measurements fail as criteria for identification.—Ascus and ascospore measurements were made from 19 original isolates of *S. sclerotiorum*, 10 of *S. trifoliorum*, and 8 of *S. minor*. It was found that there was no line of demarcation between the species studied, and certain isolates could have been placed in any 1 of the 3 species. For example, 3 isolates had very similar average ascus measurements (157.9×9.1 μ, 151.6×8.9 μ, and 157.3×11.1 μ), yet would be identified as *S. sclerotiorum*, *S. trifoliorum*, and *S. minor*, respectively, on the basis of host association and sclerotium size.

Two to 3 single-ascospore generations were obtained from original isolates, and ascus and ascospore measurements obtained from these single-ascospore progenies differed as much as those of different original isolates. For example, average ascus measurements were 145.6×7.6 μ for 1 original isolate of *S. sclerotiorum* and 153.2×8.2 μ for the third ascospore generation. However, in the first ascospore generation the ascus measurements were 129.8×8.0 μ; hence there was a difference of 23.4 μ between the average ascus length of the first and that of the third ascospore generation. These differences are much greater than the difference of 3.0–8.0μ between the average ascus lengths originally reported for *S. sclerotiorum* (22) and *S. intermedia* (20), or that of 6.0–11.0 μ between those of *S. sclerotiorum* (22) and *S. minor* (14). The average ascus measurements of the original isolates and their subsequent single-ascospore progenies tentatively identified as *S. sclerotiorum* varied from 119.0–162.4×6.4–10.9 μ and the average ascospore measurements from 10.2–14.0×6.4–7.7 μ.

Average ascus measurements obtained from original isolates and their subsequent single-ascospore progenies tentatively identified as *S. trifoliorum* varied from 151.0–195.0×8.7–10.9 μ and the average ascospore measurements from 13.7–16.3×8.0–10.0 μ. The difference between the average ascus length of an isolate and that of the first ascospore generation of another isolate was 43.8 μ, a greater difference than that of 29 μ between the average ascus length in the original descriptions of *S. trifoliorum* (11) and *S. minor* (14), or that of 25–30 μ between those of *S. trifoliorum* (11) and *S. sclerotiorum* (22).

The average ascus and ascospore measurements of original isolates and their single-ascospore progenies tentatively identified as *S. minor* were similar and represented a variation of 139.3–171.0×8.8–11.1 μ for asci, and 12.7–16.1×6.3–7.4 μ for ascospores. The difference of 31.7 μ in the average ascus length was greater than that in the original descriptions which separated *S. minor* (14) from *S. sclerotiorum* (22) or from *S. intermedia* (20). The same relative range of variation was obtained for ascus and ascospore measurements in subsequent ascospore generations as that which occurred among the original isolates.

The ascus length of the isolates tentatively identified as *S. sclerotiorum*, *S. minor*, and *S. trifoliorum* studied herein show a continuous gradation from 108.0 to 220.0 μ with no distinct break between species which would serve for purposes of identification. The measurements of the 3 species overlapped such that any isolate with ascus measurements between 130.0 and 190.0 μ could possibly fit into any 1 of the 3 species. Furthermore, the ascus-length measurement given in the description of *S. sativa* (10) overlapped that given in the description of *S. intermedia* (20), and these measurements are also overlapped by the ascus-length measurements presented herein for *S. sclerotiorum*.

There was considerable overlapping in every case of the ascospore lengths of *S. sclerotiorum*, *S. trifoliorum*, and *S. minor*. For example, isolates which produced ascospores that ranged in length from 9.6–19.5 μ could not be separated on the basis of ascospore length. The ascospore lengths given in the descriptions of *S. sativa* (10) and *S. intermedia* (20) also are within the range 9.6–19.5 μ and hence could not be distinguished from ascospores of *S. sclerotiorum*, *S. trifoliorum*, or *S. minor*.

Cultural variation in single ascospore progeny.—Single-ascospore cultures were obtained from apothecia of all original isolates as previously described, and were compared with the parent culture grown at 18–21°C on agar slants made from the same batch of agar. Each single-ascospore culture was transferred 2 or more times to potato-dextrose agar plates or slants; and if any variation was evident in the culture from a single ascospore, many transfers were made to determine if the change in type was constant

and perpetuated. Slight cultural differences were disregarded and only the more obvious ones were studied. It was found that single-ascospore progeny in almost every instance resembled the parent in cultural type, even in the third generation. However, several single-ascospore variants were obtained each of which differed markedly from its parent in certain cultural and morphological characteristics.

S. sclerotiorum.—The most pronounced variation in cultural type occurred in single-ascospore cultures from an original isolate that had been placed originally in culture Type 1. From 25 single-ascospore cultures, 18 were similar to the original isolate. Of the remaining 7 single-ascospore cultures, 4 had white aerial mycelium and very large sclerotium-like masses and 3 had very abundant brown aerial mycelium and large irregularly shaped soft punky sclerotia which were incompletely covered with the characteristic black rind. Single-ascospore cultures obtained from apothecia produced by 3 cultures of the brown-mycelium type resembled the parents.

A brown-mycelium type similar to the brown variants mentioned above was obtained from the field. When single spored, this isolate yielded cultures similar to the parent from all apothecia except 1. Single-ascospore cultures from this apothecium were

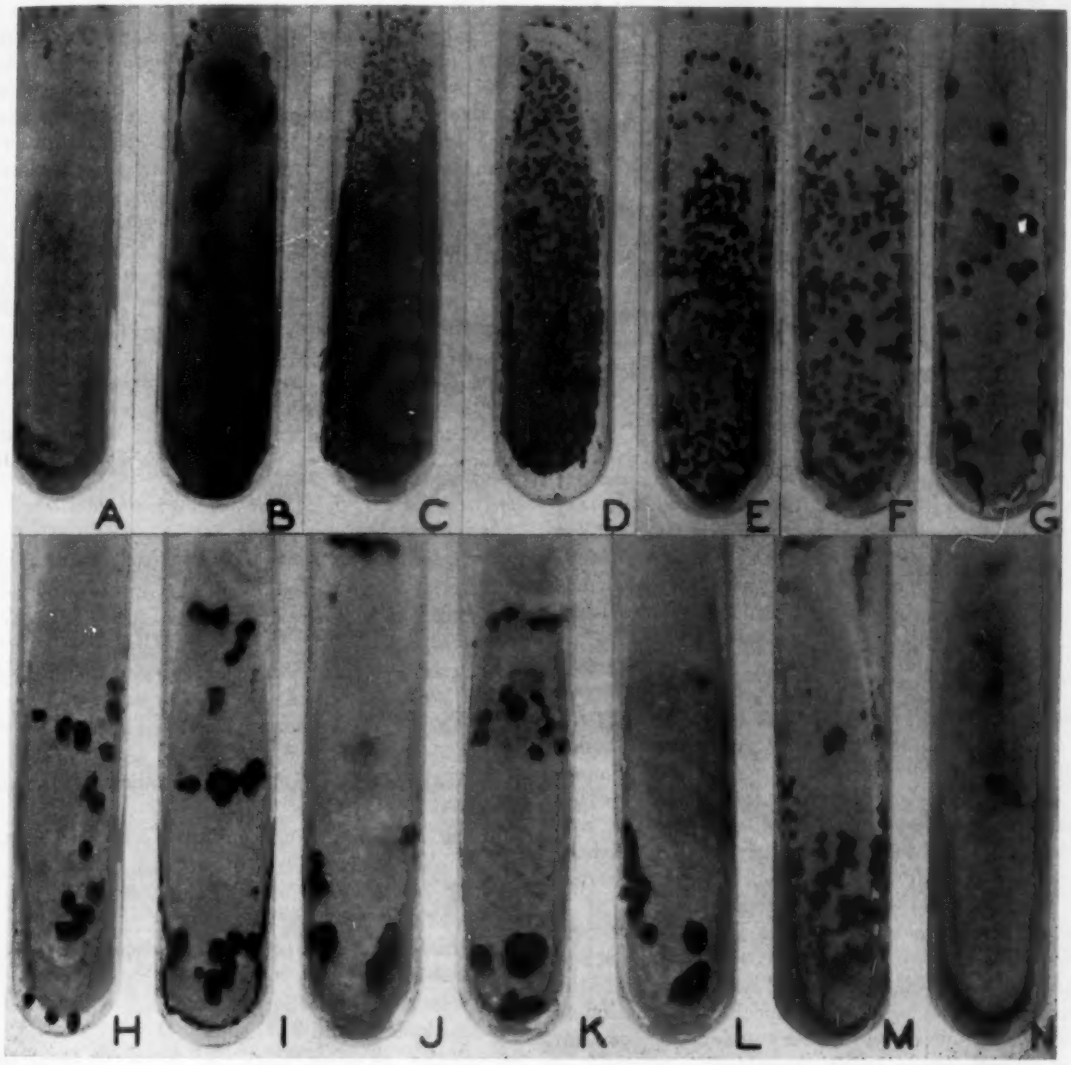

FIG. 1. Intergradation of sclerotium size among certain Sclerotinia isolates and single-ascospore cultures: A) nonsclerotial white type; B) nonsclerotial brown type; C) very-small-sclerotium type of *S. minor;* D) small-sclerotium type typical of *S. minor;* E) *S. sativa;* F) intermediate type tentatively identified as *S. minor;* G) intermediate type tentatively identified as *S. trifoliorum;* H) intermediate type tentatively identified as *S. sclerotiorum;* I) tentatively identified as *S. trifoliorum* with typical sclerotia; J) *S. sclerotiorum;* K) *S. trifoliorum;* L) *S. sclerotiorum;* M) the brown-mycelium type; and N) the large sclerotium-like mass white-mycelium type.

similar to the white-mycelium type described under culture Type 1.

A sterile white-mycelium type and a sterile brown-mycelium type that formed neither spermatia nor sclerotia were obtained from isolates which were typical of *S. sclerotiorum*. These variants of *S. sclerotiorum* were similar to the sclerotia-sterile types of *S. minor* and of *S. trifoliorum* reported by Balfe (1) and by Kreitlow (18), respectively.

S. trifoliorum.—No distinct cultural variants were observed among more than 500 single-ascospore cultures of *S. trifoliorum*. Sclerotial size varied from an intermediate size comparable to that reported for *S. intermedia* (20) to a sclerotial size of 2–7 × 2–5 mm similar to that reported for *S. sclerotiorum* (21). Appressorial masses in contact with the glass of the test tube occurred in almost all cultures. The amount of these masses varied and was not considered to be significant for purposes of identification.

S. minor.— About 200 single-ascospore cultures obtained from the 8 isolates were studied. In almost all cases, a single-ascospore culture resembled its parent in mycelial characteristics, but the sclerotia varied from approximately spherical with a diameter of less than 0.5 mm to large, irregular flattened ones with dimensions of 2–4 × 1–2 mm.

INTERGRADATION OF SCLEROTIAL SIZE.—Sclerotia of *S. minor* ranged in size from less than 0.5 mm in diameter to as large as those described for *S. intermedia* (20), *S. sclerotiorum* (21), or *S. trifoliorum* (18). Sclerotia of *S. sclerotiorum* varied from a size similar to those formed by *S. minor* (14) or by *S. intermedia* (20) to the large sclerotium-like masses formed by the white-mycelium types of *S. sclerotiorum* reported herein. Sclerotia of *S. trifoliorum* were similar to those of *S. sclerotiorum*. Sclerotial size of individual isolates was fairly constant, but there was considerable variation among different isolates of the same species. There was an intergradation (Fig. 1) in sclerotium size from the nonsclerotium types to the large sclerotia of *S. sclerotiorum* with the intervals between filled in by *S. minor*, *S. sativa*, and *S. trifoliorum*. Therefore, this character cannot be depended on with any degree of certainty for species determination. For example, isolates which form sclerotia of an intermediate size (2–4 × 2–3 mm), may produce asci and ascospores which are similar in size to those reported for *S. sclerotiorum* (9, 21), *S. trifoliorum* (11), *S. minor* (14), or *S. intermedia* (20).

DISCUSSION.—The review of the literature concerning measurements of asci and ascospores summarized in Table 1 and the data presented herein illustrate the impracticability of assigning a specific designation to the species considered herein under the present system. Furthermore, fungi should be classified at the species level "for what they are, not for what they do or where they occur" (24).

Bisby (4, p. 42) has pointed out that the taxonomist has 2 aims: "1) to arrange his categories of species, genera, families and so on so as to facilitate identification, and 2) to attempt a 'natural' arrangement". Under the present taxonomic arrangement of the species considered herein, neither of these aims has been achieved. Identification is not facilitated, and natural arrangement has not been attained.

Article 4 in the International Rules of Botanical Nomenclature (4, p. 60) states that, "The essential points in nomenclature are: 1) to aim at fixity of names; 2) to avoid or to reject the use of forms and names which may cause error or ambiguity or throw science into confusion. Next in importance is the avoidance of all useless creation of names."

S. sclerotiorum, *S. trifoliorum*, *S. trifoliorum* var. *fabae*, *S. intermedia* Ramsey, *S. minor*, and *S. sativa* constitute a natural group characterized by the presence of true sclerotia, i.e. consisting only of fungus tissue, and by the absence of conidia. Previously suggestions have been advanced that *S. trifoliorum* and *S. minor* may be synonymous (25) and that *S. intermedia* and *S. minor* differ from *S. sclerotiorum* only in characters that "cannot be considered as possessing specific value" (6). It has been shown herein that there is continuous variation in sclerotium size and in ascus and ascospore dimensions such that within this group it is impossible to set up definite species limits within which all isolates can be placed with any degree of certainty. It is therefore proposed that the 5 above-named species and the variety of *S. trifoliorum* be placed in synonymy and that on the basis of priority the binomial *Sclerotinia sclerotiorum* (Lib.) D By. apply to small-, intermediate-, and large-sclerotium isolates regardless of the host involved, as follows:

Sclerotinia sclerotiorum (Lib.) D By.

Synonyms:

Peziza sclerotiorum Lib., Cryt. Ard. No. 326. 1837.

Peziza sclerotii Fckl., Bot. Ztg. 35: 249. 1861.

Peziza ciborioides Hoffm., Icon. Anal. Fung. 3: 65. 1863.

Peziza coemansii Kickx, Fl. Cryto. Fland. 1: 485. 1867.

Peziza Kauffmanniana Tichomirow, Bul. Soc. Nat. Moscow 61: 295. 1868.

Sclerotinia libertiana Fckl., Symbolae Mycologicae 331. [1870].

Rutstroemia homocarpa Karst., Mycol. Fenn. 1: 107. 1871.

Sclerotinia ciborioides Rehm, Ascom. No. 107. 1872.

Phialea sclerotiorum Gill., Discom. France 98. 1879.

Sclerotinia trifoliorum Ericks., Kgl. Landtbr.-Akad. Handl. Tid. 19: 28. 1880.

Peziza postuma Berk. & A. S. Wilson, Gard. Chron. 20, N.S.: 333. 1883.

Sclerotinia sclerotiorum (Lib.) D By., Vergl. Morph. Biol. der Pilze, Mycet. Bact. 216. 1884.

Hymenoscypha sclerotiorum Phill., Man. Brit. Discom. 115. 1887.

Sclerotinia minor Jagger, Jour. Agr. Res. 20: 331. 1920.

Sclerotinia intermedia Ramsey, Phytopathology 14: 323. 1924.

Sclerotinia trifoliorum Ericks. var. *fabae* Keay, Ann. Appl. Biol. 26: 227. 1939.

Sclerotinia sativa Drayt. & Groves, Mycologia 35: 517. 1943.

Description: Apothecia 1 to many from a single sclerotium, funnel shaped to discoid to reflex, 0.5 mm to 2 or more cm in diameter, light to dark in color. Asci 8 spored, cylindrical to cylendro-clavate 81.0 to 252.2 by 4.3 to 22.4 μ. Ascospores eliptical to ovoid, hyaline, unicellular, 6.0 to 28.2 by 2.0 to 15.2 μ. Paraphyses filiform, hyaline. Spermatia globose. Sclerotia, if present, punky to hard, black exterior, white to pink interior, minute to several cm in size. Mycelium white to chocolate brown.

Habitat: Attacks a wide variety of monocotyledenous and dicotyledenous hosts in the field and in storage and has been reported from almost all parts of the world, but is mainly a temperate zone inhabitant.

When situations arise in which it is of some practical convenience to designate sclerotium size, it is proposed that the horticultural variety be employed for such subspecific designations as suggested by Snyder and Hansen (24). For example, the former species *S. minor* would be referred to as *S. sclerotiorum* "Minor" or *S. sclerotiorum* "Intermedia," depending on the particular interpretation of the sclerotium size. Large-sclerotium types would be referred to as *S. sclerotiorum* "Major".

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LITERATURE CITED

1. Balfe, Ilma G. 1935. An account of sclerote-forming fungi causing diseases in Matthiola, Primula, and Delphinium in Victoria. Roy. Soc. Vict., Proc. **47**: 369-386.
2. Beach, W. S. 1921. The lettuce "drop" due to Sclerotinia minor. Pa. Agr. Exp. Sta. Bul. 165, 27 p.
3. Bisby, G. R. 1924. The sclerotinia disease of sunflowers and other plants. Sci. Agr. **4**: 381-384.
4. Bisby, G. R. 1945. An introduction to the taxonomy and nomenclature of fungi. Imp. Mycol. Inst., Kew, Surrey. 117 p.
5. Chivers, A. H. 1929. A comparative study of Sclerotinia minor Jagger and Sclerotinia intermedia Ramsey in culture. Phytopathology **19**: 301-309.
6. Cunningham, G. H. 1927. Fungus diseases attacking artichokes. Incidence, life-history, and remedial treatment. New Zeal. Jour. Agr. **34**: 402-408.
7. Davis, W. H. 1925. Drop of Chinese cabbage and our common cabbage caused by Sclerotinia sclerotiorum (Lib.) Massee (Sclerotinia libertiana Fukl.) Phytopathology **15**: 249-259.
8. De Bary, A. 1884. Vergl. Morph. Biol. der Pilze, Mycet. Bact. 216.
9. Dickson, F. 1930. Studies on Sclerotinia sclerotiorum (Lib.) De Bary. Ph.D. Thesis, Cornell University, Ithaca, N. Y.
10. Drayton, F. L., and J. W. Groves. 1943. A new Sclerotinia causing a destructive disease of bulbs and legumes. Mycologia **35**: 517-528.
11. Eriksson, J. 1880. Om Klöfverrötan med särskildt afseende pá dess uppträdande i várt land under áren 1878-79. Kgl. Landtbruks-Akad. Handl. Tid. **19**: 28-42.
12. Frandsen, K. J. 1946. Studier over Sclerotinia trifoliorum Eriks. Det Danske Forlag. Copenhagen. 220 p. (English summary).
13. Held, V. M., and C. M. Haenseler. 1953. Cross-inoculations with New Jersey isolates of Sclerotinia sclerotiorum, S. minor and S. trifoliorum. U. S. Dept. Agr. Pl. Dis. Rptr. **37**: 515-517.
14. Jagger, I. C. 1920. Sclerotinia minor, n. sp., the cause of a decay of lettuce, celery, and other crops. Jour. Agr. Res. **20**: 331-334.
15. Keay, Margaret A. 1939. A study of certain species of the genus Sclerotinia. Ann. Appl. Biol. **26**: 227-246.
16. Kheswalla, K. F. 1934. Stem rot of tobacco caused by Sclerotinia sclerotiorum (Lib.) De Bary. Indian Jour. Agr. Sci. **4**: 663-673.
17. Kreitlow, K. W. 1949. Pathogenicity of Sclerotinia trifoliorum to some forage legumes. (Abs.) Phytopathology **39**: 11.
18. Kreitlow, K. W. 1949. Sclerotinia trifoliorum, a pathogen of ladino clover. Phytopathology **39**: 158-166.
19. Parievskaya, A. P. 1931. Wet or white rot of flax caused by the fungus Sclerotinia libertiana Fuckel. (In Russian). Materials for Mycol. and Phytopath. [Leningrad] **8**: 165-170. (Abs. in Rev. Appl. Mycol. **11**: 45.
20. Ramsey, G. B. 1924. Sclerotinia intermedia n. sp. a cause of decay of salsify and carrots. Phytopatholgy **14**: 323-327.
21. Ramsey, G. B. 1925. Sclerotinia species causing decay of vegetables under transit and market conditions. Jour. Agr. Res. **31**: 597-632.
22. Saccardo, P. A. 1889. Sylloge fungorum. **8**: 196.
23. Snyder, W. C., and H. N. Hansen. 1954. Concepts of species and nomenclature evolved from studies in the genus Fusarium. In Rapports et Communications. VIII Cong. Internatl. de Bot., Sec. 18-20 (Paris). p. 171-172.
24. Snyder, W. C., and H. N. Hansen. 1954. Variation and speciation in the genus Fusarium. N. Y. Acad. Sci. Ann. **60**: 16-23.
25. Valleau, W. D., E. N. Fergus, and L. Henson. 1933. Resistance of red clovers to Sclerotinia trifoliorum Erik., and infection studies. Ky. Agr. Exp. Sta. Bul. 341: 113-131.
26. Wolf, F. A., and R. O. Cromwell. 1919. Clover stem rot. N. C. Agr. Exp. Sta. Tech. Bul. 16, 22 p.
27. Young, P. A., and H. E. Morris. 1927. Sclerotinia wilt of sunflowers. Mont. Agr. Exp. Sta. Bul. 208, 32 p.

THE INFLUENCE OF CERTAIN ENVIRONMENTAL FACTORS ON EXPRESSION OF RESISTANCE TO BUNT IN WHEAT [1]

R. B. Griffith,[2] F. P. Zscheile, and J. W. Oswald

SUMMARY

Postemergence environment had a pronounced effect on the relative resistance of wheat to bunt, caused by *Tilletia caries* (DC.) Tul. Only 21 per cent of the Baart (susceptible variety) plants and none of the Baart 38 (resistant variety) plants, although heavily invaded by smut mycelium when transplanted to the field in May, were bunted at maturity. Comparable Baart plants grown to maturity in the laboratory, with a 20-hour light-period from fluorescent lights, were 100 per cent bunted in repeated experiments. No bunt balls were found on any Baart 38 plants grown under these conditions, but chlamydospores were detected microscopically within normal-appearing seeds. Ninety per cent of the inoculated Baart plants grown to maturity at 10–13°C with a 20-hour light-period from incandescent bulbs were bunted. Under these conditions 58 per cent of the inoculated Baart 38 plants developed visible manifestations of infection at maturity.

Variations were noted in growth of the inoculated Baart and Baart 38 plants under the various artificial environments. No variations were noted in field-grown plants. Under warm conditions and fluorescent lights, inoculated Baart and Baart 38 plants were stunted in early stages of growth; at maturity the inoculated Baart plants were shorter than the controls and the inoculated Baart 38 plants were equal in height to the controls. When grown at 10–13°C under incandescent lights, the inoculated Baart plants were equal in height to the controls during early stages of growth but were shorter at maturity; the inoculated Baart 38 plants were slower growing, slower maturing, and shorter at maturity than were the controls.

Resistance in Baart 38 was shown to be connected with an inhibition of chlamydospore development rather than with an inhibition of vegetative growth of the fungus within the wheat plants, as formerly supposed.

Extreme variability exists in the infection level (as measured by bunt-ball formation) of *Tilletia caries* (DC.) Tul. in field-grown susceptible varieties of wheat. This variability is apparent from year to year, in plantings made at different times in the same year, and in adjacent rows planted at the same time. In studies concerned with the nature of resistance of certain wheat varieties to *T. caries*, this variability presented a complicating factor. The results presented here were obtained during a search for controlled experimental conditions which would permit an expression of complete susceptibility in the susceptible variety and complete resistance in the derived resistant line. A preliminary account of the work has been published (2).

Reichert (7) pointed out that workers in different areas using the same host varieties and apparently the same collections of the bunt fungus have obtained different reactions. He suggested that this may be due to the influence of climatic factors on the pathogen, but did not rule out the possibility that environment might affect the capacity of the host to resist the pathogen. Rodenhiser and Holton (8) considered that environmental factors affect the host rather than the pathogen. Pugsley (5) found expression of resistance to bunt partially dependent on environmental conditions.

Faris (1) concluded that postemergence environment (following infection) had little effect on the percentage of bunted plants. Smith (12) studied the effect of temperature on the reaction to bunt of the wheat variety Hope in an attempt to explain the field observation that this variety is resistant to all races of bunt when spring-grown but susceptible when winter-grown. He found that Hope was almost completely resistant when grown to emergence at 9°C and then transferred to 21°C, whereas it was completely susceptible if grown to maturity at 9°C.

Rodenhiser and Taylor (10) concluded that variation in the interrelationship between soil type and incubation temperature may account for seasonal differences in response of a variety to a certain race of the smut fungus and for inconsistencies in the reaction of a variety when tested at different places. The same authors (9, 11) reported an effect of day-length on bunt in wheat. In general, the use of a 24-hour light-period increased the percentage of bunt. This effect was definite with some races, but with other races there was practically no effect on the level of infection.

MATERIAL AND METHODS.—Baart was used as the susceptible variety and Baart 38 as the resistant variety. Baart 38 was derived by Briggs (15) from Baart by the backcross method. These varieties are

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essentially isogenic (14) except for specific characters transferred. The gene for resistance, MM, was obtained from the variety Martin. The race of *Tilletia caries* was T-1.

The bunt balls used for inoculation were obtained from a single infected plant of Baart. To decrease variability within a given experiment, single-plant lines of the susceptible and resistant varieties were used (different single-plant lines were used in different experiments). In all experiments, inoculated and noninoculated plants of each variety were grown.

Seeds and bunt balls were surface sterilized under vacuum with 5 per cent sodium hypochlorite solution for 10 minutes. After a thorough washing in sterile water, 6 bunt balls were placed in a sterile 6-in. test tube and crushed. Fifty moist seeds, also thoroughly washed with sterile water, were then placed in the test tube; then the tube was plugged with sterile cotton and shaken. This method resulted in a heavy deposition of chlamydospores on the moist seed.

In the usual planting procedure, pots or flats were filled to within 2 in. of the top with air-dry soil, water was added to bring the soil to field capacity, and an aluminum foil cap was placed over the top prior to sterilization in an autoclave at a pressure of 15 lb. per sq. in. for 3 hours. After cooling to room temperature, seeds were placed on top of the soil and covered with 2 in. of vermiculite (an inert micaceous flaky mineral) which had been brought to its maximum water-holding capacity prior to sterilization under the same conditions. The aluminum foil was replaced and left on the pots or flats until emergence of the seedlings. This prevented moisture loss during germination and watering was not necessary until several days after emergence.

The germination temperature for all experiments was 10°C, a favorable temperature for infection of the young seedlings (3). Following emergence of the coleoptile tip from the vermiculite, the plants were removed immediately from the cold room, or were left in the cold room for several days. In the latter case, light was provided for 12 hours per day from 100-watt blue incandescent lights placed 20 in. from the surface of the vermiculite. The light intensity was about 200 ft.-candles and the air temperature during the light period was 13°C at the surface of the vermiculite.

These methods of inoculation and germination resulted in a very uniform high level of initial infection, as indicated by examination of coleoptiles stained with dilute cotton blue (6).

Soon after emergence the seedlings were placed in 1 of 4 environmental conditions: 1) a warm room, 2) the cold room in which the plants germinated, 3) the greenhouse (little control of temperature and none of light-period), or 4) the field. The plants grew to maturity under these conditions.

The warm room had forced-air ventilation but no control of temperature. Light was provided by a panel of eight 40-watt fluorescent tubes, mounted on a 27 × 50-in. panel of plywood painted with aluminum paint. White and daylight tubes were mounted alternately on the panel. The light intensity was 250 ft.-candles 2 ft. from the panel and 900 ft.-candles near the tubes. The height of the panel was adjusted to give an intensity of about 500 ft.-candles near the top of the plants during most of their growth. The light period was 20 hours. The temperature averaged about 22°C with extremes of 18° and 30°C for short periods.

Plants grown to maturity in the cold room (10°C) were under blue incandescent lights for 16 days after emergence. These lights were then replaced with 200-watt clear incandescent lights and a 20-hour light-period was used instead of one of 12 hours.

In the search for fungus mycelium or chlamydospores in plants beyond the seedling stage, a dilute cotton blue stain (6) was used as an aid in the determination of the extent of initial infection in coleoptiles. Samples also were taken of the growing point or head region of the plants at various stages of growth. These samples were killed in Karpechenko's solution (6) and run up to 70 per cent ethanol. Johansen's tertiary butyl alcohol method (4) was used for final embedding in 56–58°C tissuemat. Transverse and longitudinal sections, cut 12–20μ thick, were stained with magdala red-light green (6). In all cases tissues from noninoculated and inoculated plants were mounted on the same slides for comparison purposes.

In most experiments bunt counts were made when the seeds were in the late dough stage. A plant was considered bunted if a single bunt ball was found. Each seed was examined individually and in most instances was cut open in a visual search for chlamydospores.

RESULTS.—*Chlamydospores detected microscopically in resistant plants grown in warm room.*—In early experiments in which plants with coleoptiles emerging were moved from the cold room to the warm room, leaves emerged with difficulty from the coleoptiles of inoculated plants and in some cases mortality resulted. No such difficulty was observed in the case of noninoculated plants. Since this difficulty was also noticed in field-grown plants, it may be common with inoculated plants, but perhaps it was intensified by these experimental conditions.

In some of these plants, the coleoptiles were so tightly curled that the leaves were unable to emerge from the tip of the coleoptile in the normal manner. In extreme cases only the tip of the first true foliage leaf emerged from the tip of the coleoptile. The remainder of this leaf, other leaves, and the stem remained for a time within the coleoptile. As growth progressed, the developing tissues were compressed until sufficient pressure was developed to split the coleoptile and permit leaf emergence. Frequently this split occurred near the base of the coleoptile below the surface of the soil or vermiculite. Then because the food reserves in the seed often were

exhausted before the leaves could reach the soil surface and photosynthesize food to support growth, the plant died. Plants also were found in which the pressure and/or distortion had resulted in a complete rupture of the stem. All gradations occurred between this extreme and the other in which only the tip of the first leaf was retained by the coleoptile but the remainder of this leaf and the other leaves split the coleoptile below the tip and emerged more or less normally. In the latter instance very little damage to the plant was apparent.

Chlorotic flecking and malformation of the leaves (Fig. 1) were found occasionally in noninoculated plants but were much more prevalent in the inoculated plants (Table 1). This flecking condition seldom was found in any but the first 2 or 3 leaves of the plant and was most commonly associated with vascular bundles, although mesophyll areas also were affected.

In later experiments it was found that these difficulties could be eliminated by leaving the plants in the cold room until after the first 2 or 3 leaves had emerged from the coleoptile.

Inoculated plants of both resistant and susceptible varieties were much smaller in early stages of growth than were the comparable noninoculated plants. In later stages of growth the inoculated resistant plants became as large at maturity as the noninoculated plants of both varieties. The inoculated susceptible plants, however, remained smaller than plants of the other 3 classes throughout their growth cycle. There was little difference in time of maturity among the 4 classes of plants (susceptible and resistant, inoculated and noninoculated).

Table 1.—*Effect of Tilletia infection on susceptible and resistant wheat plants grown for 14 days in warm room after emerging in the cold room*

	Susceptible (Baart)		Resistant (Baart 38)	
	Noninoculated	Inoculated	Noninoculated	Inoculated
Total plants	61	153	60	153
Plants with tight coleoptile	1	119	1	105
Plants with spotted chlorotic leaves	0	86	0	103
Dead plants	0	2	0	4
Plants with malformed leaves	1	53	0	37
Average distance from seed to leaf tip (cm)	42	27	32	23

At maturity every inoculated Baart plant (a total of 159 in 3 experiments) that produced a head also produced bunt balls and was classed as bunted. On the other hand, no bunt balls were found on any of 142 inoculated Baart 38 plants which produced heads. One hundred eight Baart and 103 Baart 38 noninoculated plants were grown to provide adequate comparison between infected and noninfected plants. A composite summary of the results in 2 of these 3 experiments is given in Table 2. Under these conditions there was complete susceptibility of the susceptible

FIG. 1. Wheat seedlings illustrating leaf abnormalities caused by Tilletia infection. Left—Normal resistant Baart 38 plant on left and an inoculated one with tight coleoptile on the right. The individual leaves had the chlorotic flecking characteristic of inoculated plants. Right—two inoculated susceptible Baart plants with tight coleoptiles.

TABLE 2.—*Harvest and disease data on inoculated and noninoculated susceptible and resistant plants grown to maturity in warm room*

	Susceptible (Baart)		Resistant (Baart 38)	
	Noninoculated	Inoculated	Noninoculated	Inoculated
Number of plants harvested	46	60	44	57
Number of bunted plants	0	60	0	0
Average number of bunt balls per plant	0	6.7	0	0
Average number of seeds per plant	2.9	0	3.1	4.2
Average height of plants (cm)	38	33	38	41

Baart variety and apparently complete resistance of Baart 38.

In this experiment the presence of the fungus could be demonstrated easily in the coleoptiles of practically all inoculated plants of both the susceptible and resistant varieties after emergence at a low temperature. In most cases typical fungus mycelium also could be found in the first true foliage leaf. It was thought that differences with respect to the presence of mycelium would be evident between the susceptible and resistant varieties in the early stages of growth; therefore few samples were taken in this experiment as the plants approached maturity.

Since it had been assumed that the fungus growth probably was suppressed at the time the inoculated Baart 38 plants grew out of their early stunted condition, it was a surprise to find chlamydospores in the centers of seeds from 2 of the 3 Baart 38 plants examined microscopically in the advanced heading stage (Fig. 2, C, D). It is evident (Fig. 2) that more of the volume of the kernel is occupied by spores in the case of Baart than in that of Baart 38 and that more of these are mature than in Baart 38. This difference is presumably due to some measure of resistance still being expressed by Baart 38.

Although each seed from 142 other plants grown under these conditions was examined individually for chlamydospores, usually by cutting the seed open, no chlamydospores had been detected macroscopically. Further work is in progress to determine the prevalence of chlamydospore formation in the seeds of inoculated resistant plants.

Break-down in the resistance of Baart 38 when plants are grown to maturity in cold room.—In contrast with growth in the warm room, the height of the inoculated plants of both varieties in the cold room was the same as that of the noninoculated ones in early stages of growth (Fig. 3). Close examination of the plants of both varieties from the cold room showed shorter internodes on the inoculated Baart 38 plants. In later stages of growth the inoculated Baart 38 plants lagged behind the other 3 classes of plants and matured much later. Since the noninoculated Baart 38 plants matured at the same time as the Baart plants, the delay in maturity of the inoculated plants was presumably a response of the resistant variety to infection under this set of environmental conditions.

All plants were flowering 113 days after emergence of the seedlings. At this time the inoculated Baart 38 plants were distinctly shorter than those of the other groups, and were just coming into full flower. Completely and partially blasted heads were visible on some of the inoculated Baart 38 plants and an examination macroscopically and microscopically of 10 ovules from as many plants showed the development of chlamydospores and immature bunt balls. The plants were allowed to mature and were harvested 138 days after emergence, when the disease records were taken (Table 3). It is interesting to note that at harvest the noninoculated plants of the 2 varieties were equal in height and that they were appreciably taller than the corresponding inoculated plants.

Ninety per cent of the inoculated Baart (susceptible) and 58 per cent of the inoculated Baart 38 (resistant) plants with heads produced bunt balls or blasted heads. Unfortunately there was almost no seed production on the noninoculated plants. This complicated numerical interpretation of the results. The 3 heads of inoculated Baart which were not bunted (though empty of seed) (Table 3) may have been on tillers produced on plants sampled for histological examination, plants which had not retained their primary shoots. The bunt balls found on Baart 38 plants were smaller than those on Baart and are suggestive of the atypical bunt balls described by Smith (13) on the variety Martin infected with race T-2 of the bunt fungus. Thus there was a severe breakdown in the resistance of the resistant variety, and for the first time a macroscopic destruction of tissues (blasted heads) was evident.

TABLE 3.—*Harvest and disease data on inoculated and noninoculated susceptible and resistant plants grown to maturity in the cold room. Plants were harvested 138 days after emergence*

	Susceptible (Baart)		Resistant (Baart 38)	
	Noninoculated	Inoculated	Noninoculated	Inoculated
Total number of plants	32	31	33	31
Average Height (cm)	80.5	66.9	77.3	68.9
Total number of heads	32	31	32	29
Heads with bunt balls		28		15
Heads with whole seed	0	0	1	2
Completely empty heads	29	3	29	13
Percentage of heads bunted		90		52
Total number of blasted heads	0	0	1	3
With bunt balls	0	0	1	1
Without bunt balls	0	0	0	2
Total number of whole seeds	0	0	1	2
Total number of bunt balls		254		47

Partial breakdown in resistance of Baart 38 obtained in the greenhouse.—Among the plants grown in the greenhouse after emergence in the cold room, the effects of infection on growth characteristics were similar to those obtained in the cold room, and some plants of inoculated Baart 38 were bunted.

In the first experiment under these conditions, the plants were grown to maturity in the greenhouse. At the 1- to 2-leaf stage, mycelium was found abundantly in the coleoptiles of 4 inoculated plants of each variety. At maturity, 16 of 20 inoculated Baart plants were bunted. No bunted plants were found in the other 3 classes of plants.

In the second experiment, plants were grown in the

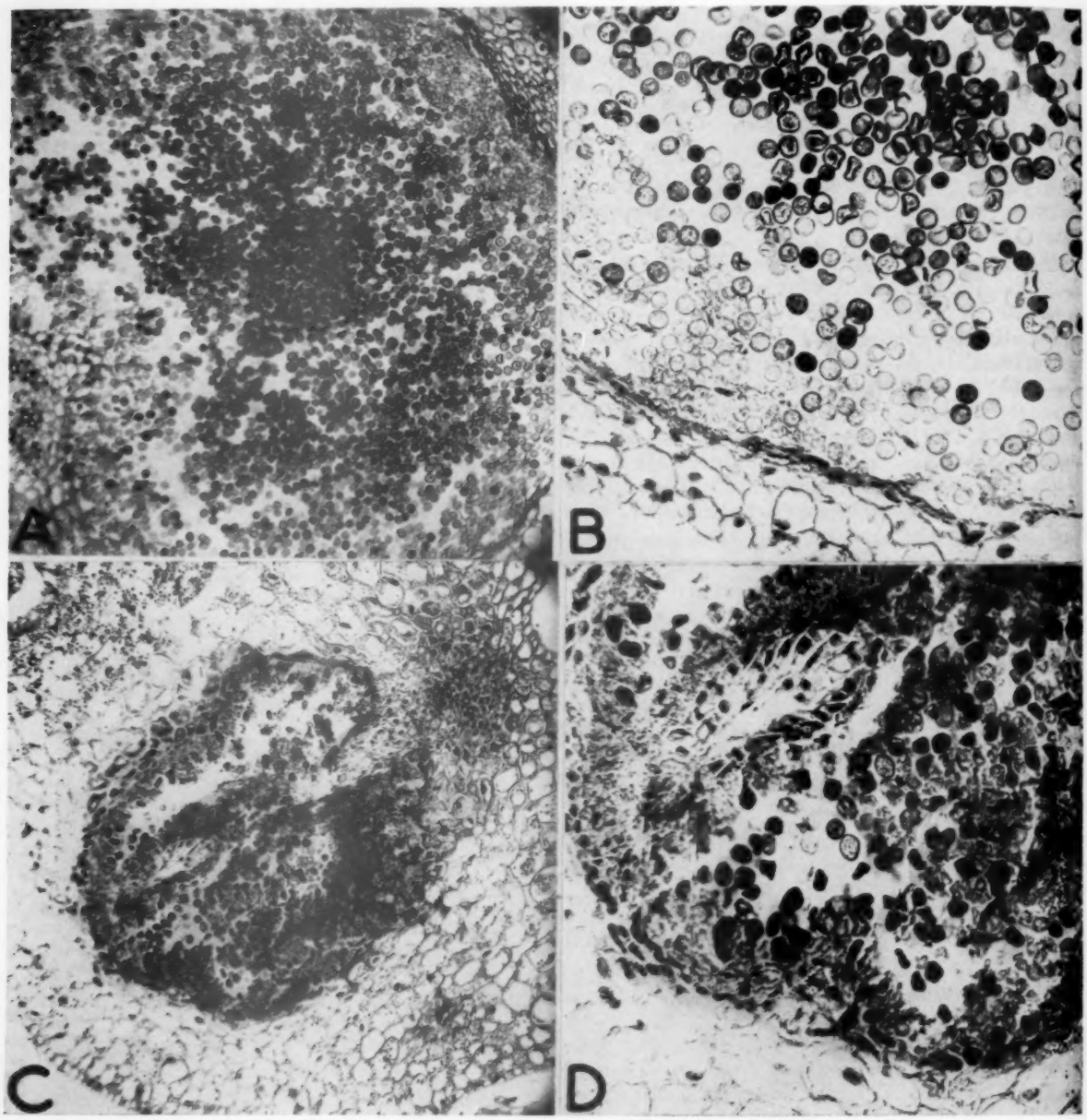

FIG. 2. A microscopic comparison of chlamydospore formation in susceptible and resistant kernels in the warm room. Cross-section views of chlamydospores found in comparable kernels from wheat plants, 50 days after emergence. A and C indicate the relative maximum sizes of the spore areas and abundance of chlamydospores in susceptible and resistant strains, respectively. B and D present more detail at higher magnification. A) Baart "kernel," recognizable as a black bunt ball. Note the large number of mature chlamydospores. Magnification 125. B) Same as A, but magnification 400. C) Baart 38 kernel, with normal healthy exterior appearance. Chlamydospores were not visible until the section was stained. Note the smaller total number of chlamydospores and the immaturity of most of them. Magnification 125. D) Same as C, but magnification 400.

warm room for 11 days and then transferred to the greenhouse. At the time of transfer to the greenhouse the inoculated plants were very small and weak. The inoculated Baart plants recovered rapidly and were soon equal in growth to the noninoculated plants. The inoculated Baart 38 plants grew more slowly and matured about 3 weeks later than the other 3 classes of plants. This growth pattern for the inoculated Baart 38 plants also was noted in the first greenhouse experiment and in the cold room.

At maturity all of 15 inoculated Baart plants were well bunted. On each of 2 of the 17 inoculated Baart 38 plants, a single bunt ball was produced.

The growth response and partial breakdown in the resistance of inoculated Baart 38 plants in the greenhouse are of special interest in that they are intermediate between those responses resulting from growth in the warm and cold rooms.

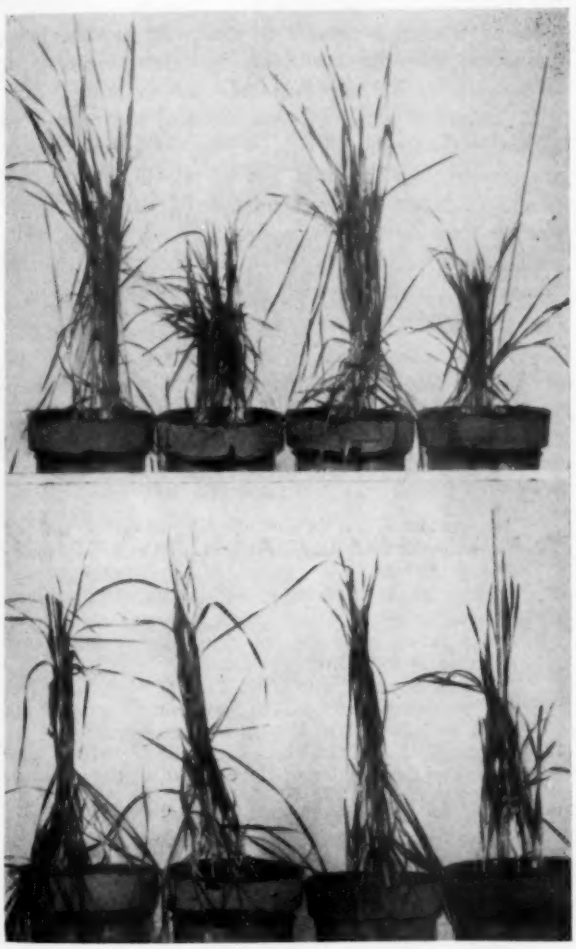

FIG. 3. Comparative growth of infected and noninfected plants 32 days after emergence of plants from vermiculite. Pots left to right in each picture: noninoculated Baart (susceptible), inoculated Baart, noninoculated Baart 38 (resistant), and inoculated Baart 38. Infection reduced height in the warm room (upper), but not in the cold room (lower).

Low bunt count obtained in field-grown Baart.—It has been generally assumed that a low percentage of bunted plants in a susceptible variety grown in the field is caused by failure of some of the plants to become infected. If this is true, production of susceptible seedlings under conditions that permit a very high and uniform level of initial infection, with no escapes, should result in all plants being bunted when transplanted and grown in the field.

To test this, seeds were inoculated as previously described and sown in flats. At the time the seedlings emerged, the plants were removed from the cold room and after a conditioning period in the laboratory, were transplanted to the field on May 23, 1951. As a consequence of the late planting, the plants were grown abnormally late in the season. At the time of transplanting, a large percentage of the inoculated plants of both varieties had the tight coleoptile which undoubtedly contributed to a rather high mortality. Histological examination of the plants at this time showed a very high and uniform level of initial infection among the inoculated plants of both varieties.

Growth rates for all 4 classes of plants were very similar, and no significant growth differences were noted among the 4 classes at maturity. Each plant was harvested separately and each kernel was examined closely for bunt balls. Bunt balls were detected on only 9 out of 42 inoculated Baart plants and none were found on the other 3 classes of plants. On these 9 plants, 8 totally bunted heads and 4 partially bunted heads were found among a total of 34 heads. The main tiller was bunted in only 2 of the 9 plants. The low percentage of bunted plants indicates a relatively high level of "resistance" even in the susceptible plants when grown under this particular set of environmental conditions. Forty-six comparable inoculated Baart plants grown simultaneously to maturity in the warm room were completely bunted—a further indication that initial infection had been complete.

These results indicate that much of the variability in the percentage of bunted plants obtained in the regular bunt nurseries from year to year may be explainable on the basis of postemergence environmental factors rather than on lack of initial infection.

DISCUSSION.—This study indicates that the expression of resistance to bunt in Baart (susceptible) and Baart 38 (resistant) is influenced considerably by environmental factors. Controlled experimental conditions were found which permit an expression of complete susceptibility in the susceptible variety and complete resistance in the derived resistant line (as measured by bunt-ball formation). The experimental conditions were not sufficiently varied to permit quantitative evaluation of the environmental factors involved. Expression of the resistant factor(s) is considered to be confined to the period of fruiting of the bunt fungus. Chlamydospore formation may be completely or only partially inhibited. The finding of chlamydospores in apparently normal kernels of a

resistant variety extends the earlier observations of Woolman (16) who reported that both susceptible and resistant varieties were equally invaded by *T. caries* but that fungus could rarely be found in tissues other than those of the coleoptile and first 2 leaves. This discovery poses the question whether these internal chlamydospores are capable of bringing about infection of the plants produced by such "infected" seeds. This possibility should be checked in further work. We concur with Woolman's conclusions on the difficulty of finding the fungus mycelium in older tissues.

The behavior of Baart 38 in relation to temperature is similar to that of the variety Hope, as described by Smith (12). He reported decreased resistance to bunt when low temperatures followed emergence. Hope exhibits greater resistance when sown in the spring than when grown as a winter wheat. More recently Pugsley (5) reported completely bunted heads common on susceptible families of wheat but only partially bunted heads and kernels on resistant plants.

The differential growth response of inoculated plants of the 2 varieties varied with the environmental conditions under which the plants were grown. The slower early growth and delayed maturity of the inoculated Baart 38 plants were associated with greenhouse conditions which also resulted in some breakdown in bunt resistance. Delayed maturity of inoculated Baart 38 plants resulted also from growth at continuous low temperatures. The production of blasted heads and bunt balls in the inoculated resistant plants indicates that this slower growth and delayed maturity were probably direct effects of the fungus, resultant from an increased degree of susceptibility on the part of the host.

The apparent recovery of the inoculated Baart 38 plants during later stages of growth in the warm room may indicate an increase in the relative resistance of the plants under this set of environmental conditions. All differential growth responses noted for the inoculated plants were caused by infection, since noninoculated plants showed no such responses. Because of the wide variability found in field-grown material, controlled environmental conditions are highly desirable, and perhaps a necessity, for determining the fundamental reasons for resistance.

The present study indicates that a thorough knowledge of host-parasite-environment relationships is a necessary first step in the determination of the mechanism of resistance. Thus, the possibilities of finding a biochemical basis for resistance of wheat to bunt during the vegetative stage of growth of the wheat plants would now seem remote unless further work reveals environmental conditions that allow expression of the resistant factor(s) during vegetative stages of the wheat plant.

Expression of resistance of Baart 38 apparently is connected with an inhibition of chlamydospore development by *T. caries* in this variety rather than with lack of vegetative development of the fungus, as shown by the finding of chlamydospores in seeds which macroscopically appeared not to be bunted. Bunted plants of Baart 38 have bunt balls that are much smaller than those on comparable Baart plants, indicating a low residual expression of resistance, even in "resistant" plants on which the kernels obviously have been displaced by chlamydospores.

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LITERATURE CITED

1. Faris, J. A. 1924. Factors influencing the infection of wheat by Tilletia tritici and Tilletia laevis. Mycologia **16**: 259-282.
2. Griffith, R. B., F. P. Zscheile, and J. S. Oswald. 1953. Resistance of wheat to bunt associated with inability to produce chlamydospores. (Abs.) Phytopathology **43**: 473.
3. Holton, C. S., and F. D. Heald. 1941. Bunt or stinking smut of wheat (a world problem). Burgess, Minneapolis, Minn. 211 p.
4. Johansen, D. A. 1940. Plant microtechnique. McGraw-Hill, New York and London. 523 p.
5. Pugsley, A. T. 1953. Backcrossing of wheat for resistance to bunt in South Australia. Empire Jour. Exp. Agr. **21**: 314.
6. Rawlings, T. E. 1933. Phytopathological and botanical research methods. Wiley, New York. 156 p.
7. Reichert, I. 1930. The susceptibility of American wheat varieties resistant to Tilletia tritici. Phytopathology **20**: 973-980.
8. Rodenhiser, H. A., and C. S. Holton. 1942. Variability in reaction of wheat differential varieties to physiologic races of Tilletia levis and T. tritici. Phytopathology **32**: 158-165.
9. Rodenhiser, H. A., and J. W. Taylor. 1940. Studies on environmental factors affecting infection and the development of bunt in wheat. (Abs.) Phytopathology **30**: 20.
10. Rodenhiser, H. A., and J. W. Taylor. 1940. Effects of soil type, soil sterilization, and soil reaction on bunt infection at different incubation temperatures. Phytopathology **30**: 400-408.
11. Rodenhiser, H. A., and J. W. Taylor. 1943. The effect of photoperiodism on the development of bunt in two spring wheats. Phytopathology **33**: 240-244.
12. Smith, W. K. 1932. The effect of different temperatures on the reaction of Hope wheat to stinking smut. Phytopathology **22**: 615-627.
13. Smith, W. K. 1932. The reaction of Martin wheat to three physiologic forms of Tilletia tritici. Phytopathology **22**: 847-850.
14. Suneson, C. A. 1947. An evaluation of nine backcross-derived wheats. Hilgardia **17**: 501-510.
15. Suneson, C. A., and F. N. Briggs. 1941. Wheat production in California. Calif. Agr. Exp. Sta. Bul. 659, 18 p.
16. Woolman, H. M. 1930. Infection phenomena and host reactions caused by Tilletia tritici in susceptible and non-susceptible varieties of wheat. Phytopathology **20**: 637-652.

FUSARIUM WILT OF CUCUMBER [1]

John H. Owen [2]

SUMMARY

A destructive Fusarium wilt of cucumber causes damping-off of young seedlings and a typical vascular wilt of older cucumber plants. In cross-inoculation tests on seedlings of cucumber, watermelon, and muskmelon, this Fusarium from cucumber and one from watermelon were highly pathogenic on their original hosts. The Fusarium from cucumber was also highly pathogenic on muskmelon and slightly pathogenic on watermelon seedlings. The Fusarium from watermelon infected seedlings of some varieties of muskmelon, but not of cucumbers.

Fusarium wilt of cucumber has become well established and widespread in the Sumter County, Florida, area during the past 6 years. Weber (16) stated in 1925 that Fusarium wilt of cucumber occurred in Florida but that it was not common. It was first observed by growers in the 1948 fall crop, and the first reports of severe losses were made during the 1949 spring season. Since 1949 the disease has become progressively worse, forcing growers to move to newly cleared land after their soil had become infested with the wilt fungus. Although Fusarium wilt of cucumber has been observed previously in Florida and in other states, this is the first account of extensive losses caused by the fungus in the United States.

Selby (9) in 1898 reported a wilt of garden cucumber (Japanese climbing cucumber) and believed it to be similar to the Fusarium wilt of watermelon. In 1911 Stone (12) observed a Fusarium wilt on greenhouse cucumber in Massachusetts but made no further study of the disease. Weber (15) stated in 1924 that a Fusarium wilt of cucumber occurred in Denmark and was caused by *Fusarium vasinfectum*. In 1943 van Koot (14) described a foot rot and wilt of cucumber in Holland and characterized the Fusarium species isolated from diseased plants as "*Fusarium solani* and its var. *martii*, *F. orthoceras* and its var. *longius*, and *F. angustum*." Behr (1) in 1948 reported a wilt of cucumber in Germany caused by several species of *Fusarium*. The same year, Loest (5) observed a Fusarium wilt of cucumber in South Africa. *F. oxysporum* f. *niveum* was reported by Roll-Hansen (8) in 1949 as causing a severe wilt of greenhouse cucumbers in Norway. He also stated that *F. oxysporum* was isolated from the interior of cucumber seedlings without external symptoms. The symptoms described in most of these publications indicated that wilt was a result of cortical rot and not of a vascular Fusarium.

Results of studies of the disease as it occurs in Florida and of the pathogenicity of the fungus are given in this paper. A preliminary report has been published (7).

SYMPTOMS.—The wilt fungus attacked the cucumber plant at all stages of development. Damping-off of young seedlings was common and often very severe on heavily infested soil. Three to 5 days after emergence young infected seedlings became dull green and damped-off (Fig. 1, A). The disease was similar to that produced by many of the damping-off fungi, with the infected roots becoming tan-colored and the young seedlings toppling over and dying. Pre-emergence damping-off occurred in soil infested with the Fusarium from cucumber. Infection of older plants which had not yet developed runners usually resulted in the wilting of the entire plant (Fig. 2). On more mature plants with runners, a single crown branch occasionally wilted, followed by the wilting of the entire plant. Wilting occurred over a period of 3–5 days with the infected plant often partially reviving at night and wilting in the middle of the day during the early stages of the disease. Finally, the runner or the entire plant collapsed rapidly.

A tan discoloration of the vascular bundles often extended into the vine for 6–8 nodes (Fig. 1, B). In advanced cases, the main roots usually had a light tan discoloration.

ECONOMIC IMPORTANCE.—Approximately 800 acres of cucumbers are grown in the Sumter County area annually, mainly as an early spring crop. Observations of the disease made in 1949 by W. B. Tisdale showed that stands in some fields were so poor that they were plowed under and the fields replanted, while losses in other fields ranged from 1–40 per cent. On newly cleared land the disease appeared only in scattered spots toward the end of the season. The fungus may be introduced into the new soil from the old infested fields through the movement of equipment and laborers.

Cucumber plants grown on infested soil for the second or third consecutive season often exhibited 25–70 per cent infection by the time of the first picking. In these fields the plants in entire rows and in large sections of the field became infected and died.

PATHOGENICITY OF THE FUNGUS.—Tests were made on the pathogenicity of the Fusarium from cucumber on cucumber seedlings. Since the Fusarium from watermelon, *Fusarium oxysporum f. niveum* (E. F. Sm.) Snyd. & Hans., was the only cucurbit Fusarium wilt fungus commonly found in Florida, cross-inoculation experiments were conducted on various cucurbit hosts with this fungus and the Fusarium from cucum-

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ber in an attempt to determine the relationship of these 2 fungi. Isolates of the Fusaria from cucumber and watermelon were made from the stems of wilted cucumber and watermelon plants, respectively. Inocula containing heavy concentration of microconidia were prepared from shake-cultures grown in flasks of potato-dextrose liquid medium for 7 days at 28°C. Fifty ml of inoculum was mixed with each 6-in. pot of steam-sterilized soil. Fifteen seeds of 1 variety were planted in each of 3 pots: 1 containing soil infested with the cucumber fungus, 1 containing soil infested with the watermelon fungus, and 1 containing sterile soil. The percentage of damped-off seedlings was used as a measure of disease severity. Experiments were conducted in a greenhouse at a temperature of 20–23°C.

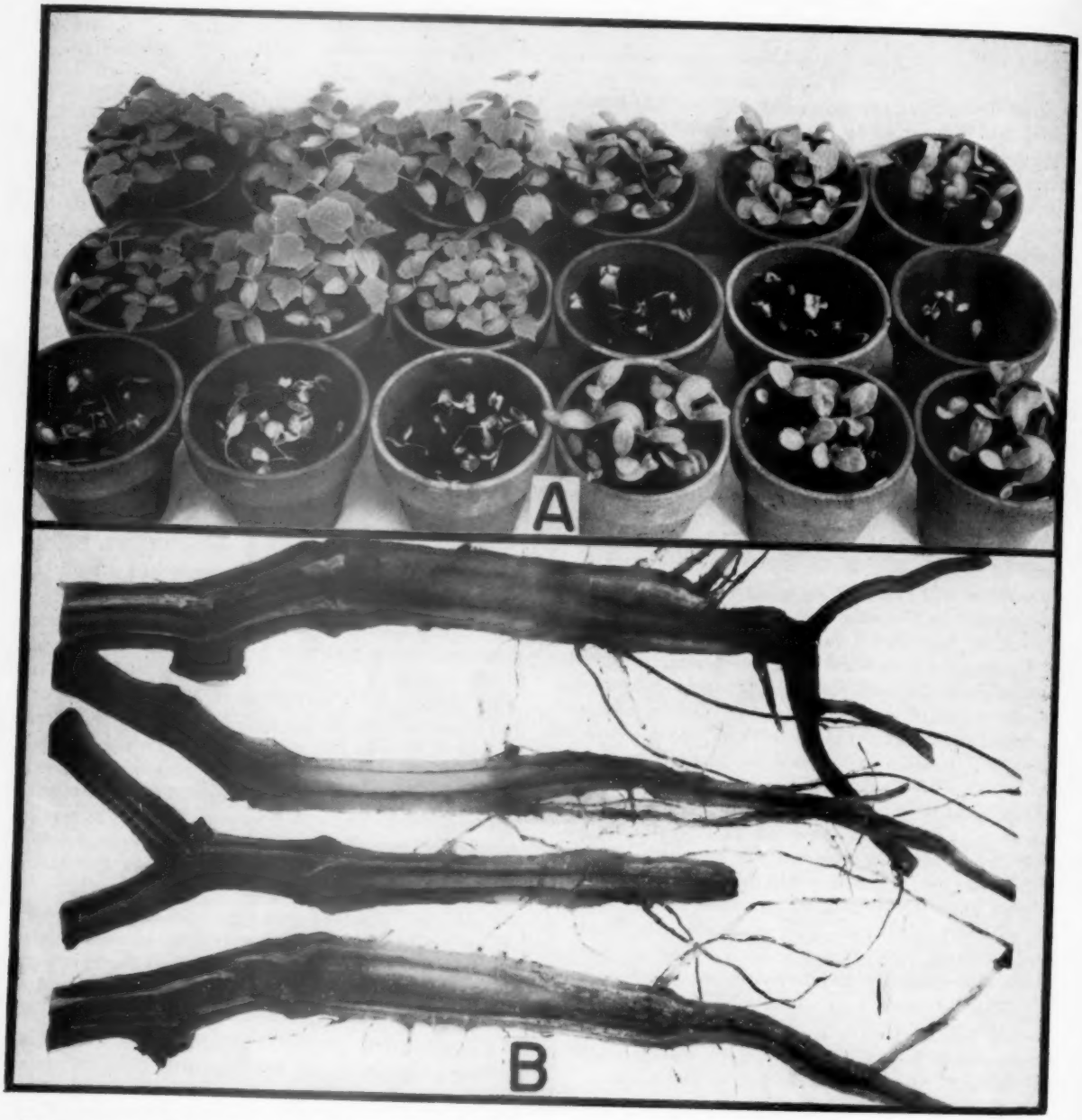

FIG. 1. A) Vertical rows 1, 2, and 3 from left to right are cucumber varieties Marketer, Palmetto, and Puerto Rico No. 39; rows 4, 5, and 6 are watermelon varieties Tom Watson, Genuine Garrison, and Stone Mountain. Horizontal rows from bottom to top: Row 1—Soil infested with the Fusarium from cucumber. Row 2—Soil infested with the Fusarium from watermelon. Row 3.—Control. B) Longitudinal sections of diseased cucumber roots, showing browning of vascular bundles extending out into stems.

FIG. 2. Fusarium wilt of naturally infected cucumber plants of the variety Marketer.

Pathogenicity tests with 7 isolates of the Fusarium from cucumber on cucumber variety Marketer indicated that there was little difference in the pathogenicity of the 7 isolates, with infection ranging from 78–100 per cent with all isolates. One of the isolates was selected to use in cross-inoculation tests with the Fusarium from watermelon on a large number of cucumber varieties and other cucurbits.

In the first cross-inoculation experiment, 22 varieties of cucumber and 13 varieties of watermelon were used: *Cucumber*—Early Fortune, Improved Long Green, Everbearing, Extra Early Green, Fordhook Famous, Chicago Pickling, Fordhook Pickling, Davis' Perfect, Burpee's Fordhook White Spine, Black Diamond, York State Pickling, Heinz Pickling, Mincee, Burpee's Cubit, Straight-8, Wauchula Stay Green Longer, Palmetto, Puerto Rico No. 39, Burpee's Sunnybrook, A and C, Burpee's Extra Early White Spine, and Marketer; *Watermelon*—Stone Mountain, Cannon Ball, Florida Favorite, Kleckley's Sweet, Congo, Dixie Queen, Blacklee, Tom Watson, Black Kleckley, Hawkesbury, Genuine Garrison, Super Black Diamond, and New Hampshire Midget.

The first damping-off of seedlings occurred within 8 days after planting. After 14 days there was a high percentage of damping-off of all cucumber varieties planted in soil infested with the cucumber fungus, except Fordhook Famous of which only 16 per cent were killed. Cucumber seedlings growing on soil infested with the Fusarium from watermelon were all healthy. The Fusarium from watermelon was highly pathogenic on all 13 varieties of watermelon, killing all seedlings of the varieties Genuine Garrison, Stone Mountain, Tom Watson, Super Black Diamond, and New Hampshire Midget. On soil infested with the Fusarium from cucumber an occasional seedling damped-off in the watermelon varieties Genuine Garrison, Florida Favorite, Blacklee, Tom Watson, Super Black Diamond, and New Hampshire Midget. The Fusarium fungus from cucumber was isolated from these damped-off seedlings.

The experiment was repeated, using the most susceptible cucumber varieties, Palmetto and Marketer; the most resistant one, Fordhook Famous; and the watermelon varieties Tom Watson and Florida Favorite. In the preceding experiment all seedlings of both watermelon varieties were infected by the watermelon fungus and 8 and 12 per cent, respectively, by the Fusarium from cucumber. Five replicates of each variety were included. In the second experiment, results (Table 1) were similar to those obtained in the first one. Thirty-eight per cent of the seedlings of Fordhook Famous were infected while Marketer and Palmetto were highly susceptible to the Fusarium from cucumber. Again there was no infection of cucumber seedlings by the Fusarium from watermelon. All of the seedlings of the watermelon varieties Florida Favorite and Tom Watson were infected by the Fusarium from watermelon. On soil infested with the Fusarium from cucumber, 33 per cent of the seedlings of Florida Favorite and 21 per cent of those of the Tom Watson variety damped-off.

In a third and fourth experiment the Fusaria from cucumber and watermelon were used in cross-inoculation experiments with cucumber, watermelon, muskmelon, pumpkin, squash, and gherkin. The procedure used was identical with that used in the preceding experiments.

The pathogenicity of the 2 Fusaria on cucumber and watermelon varieties was similar to that in previous experiments (Table 2). In the fourth experiment the cucumber variety Fordhook Famous showed pre-

TABLE 1.—*Percentage of damping-off of cucumber and watermelon seedlings on soil infested with Fusaria from cucumber and from watermelon*

Varieties	Cucumber Fusarium		Watermelon Fusarium		Noninfested control [a]
	Emerged	Damped-off	Emerged	Damped-off	Emerged
Cucumbers	per cent	per cent	per cent	per cent	per cent
Fordhook Famous	77	38	88	0	93
Palmetto	89	100	97	0	100
Marketer	97	78	64	0	93
Watermelon					
Florida Favorite	64	33	77	100	73
Tom Watson	84	21	84	100	100

[a] No damping-off occurred.

TABLE 2.—*Percentage of damping-off of cucurbits with Fusaria from cucumber and from watermelon*

Host	Cucumber Fusarium		Watermelon Fusarium		Noninfested control[a]
	Plants emerged	Damped-off	Plants emerged	Damped-off	Plants emerged
	per cent	per cent	per cent	per cent	per cent
			(Third experiment)		
Cucumber					
Marketer	96	78	97	0	100
Palmetto	89	100	48	0	93
Fordhook Famous	77	38	88	0	93
Watermelon					
Super Black Diamond	57	6	57	100	67
New Hampshire Midget	70	14	47	100	73
Florida Favorite	64	33	77	100	73
Tom Watson	84	20	83	100	100
Muskmelon					
Hale's Best No. 36	7	100	13	0	33
Hale's Best, J. S.	57	65	57	0	40
Smith's Perfect	43	100	30	100	33
Banana	80	71	57	0	75
Pumpkin					
Small Sugar	23	0	20	0	20
			(Fourth experiment)		
Cucumber					
Palmetto	93	64	100	0	100
Fordhook Famous	47	0	43	0	100
Muskmelon					
Hale's Best No. 36	0	0	30	22	67
Hale's Best, J. S.	20	100	43	0	80
Smith's Perfect	0	0	30	100	73
Banana	57	100	90	7	87
Squash					
Yellow Summer Crookneck	77	0	90	0	94
Table Queen	93	0	83	0	100
Royal Acorn	67	0	80	0	73
Pumpkin					
Small Sugar	27	0	30	0	40
Gherkin					
West Indian	27	0	33	0	33

[a] No damping-off occurred.

emergence damping-off with both fungi, but no post-emergence damping-off with either fungus. The Fusarium from cucumber was highly pathogenic on all of the muskmelon varieties tested, and pre-emergence damping-off occurred with the varieties Hale's Best No. 36 and Smith's Perfect. There was no infection on pumpkin, squash, or gherkin by either fungus. The Fusarium from watermelon infected all seedlings of the muskmelon variety Smith's Perfect in both experiments. In the third experiment there was no post-emergence damping-off in varieties Hale's Best No. 36, Hale's Best J. S. or Banana; however, in the fourth experiment some seedlings of the last 2 varieties damped-off. Some pre-emergence damping-off occurred in both experiments.

FIELD PLOT EXPERIMENT.—The Fusarium from cucumber was grown on sterile oat medium, and the inoculum was worked into the soil before seeds were planted. The soil which had not been planted to cucumbers previously was infested in the spring of 1953 with Fusarium from cucumber and planted with the seeds of the Marketer cucumber. Only an occasional seedling damped-off and no mature plants were infected. In the spring of 1954, the same soil again was infested and planted to cucumbers. This time there was a high percentage of damping-off in the seedling stage, and the cucumber wilt fungus was reisolated. Infection continued throughout the season, affecting plants during all stages of growth and resulting in the death of a high percentage of the plants.

DISCUSSION.—The Fusarium from cucumber used in the present studies is highly pathogenic on cucumber and thus appears to differ from previously reported Fusaria from watermelon and muskmelon. The forms isolated from these 2 hosts were pathogenic on their respective hosts, but not on cucumber. E. F. Smith (10) in 1894 conducted inoculation studies with the Fusarium from watermelon and stated that it was specific to watermelon. Taubenhaus (13) in 1920 published results of numerous trials of various cucurbits grown on soil infested with the Fusarium from watermelon and found that muskmelon, cucumber, squash, cushaw, citron, and gourd were not infected while 69–98 per cent of the watermelon plants were killed. In 1937 Leach and Currence (4), studying Fusarium wilt of muskmelon in Minnesota, reported

that the Fusarium from watermelon would not infect muskmelon, and the Fusarium from muskmelon would not attack watermelon. In 1943 van Koot (14) described a Fusarium wilt of cucumber in Holland and isolated 4 *Fusarium* spp. from the stems of wilted plants. Two of these, *F. angustum* and *F. orthoceras*, were found to produce a vascular wilt and a brown discoloration of the crown. *F. orthoceras* var. *longius* and *F. solani* attacked the crown and roots producing a cortical type rot. In cross-inoculation tests on seedlings, all 4 *Fusarium* spp. were found to be pathogenic on cucumber and muskmelon. In 1944, Cox (2) reported that the plants remained healthy when muskmelon and cucumber scions were grafted on to cucumber stocks growing in soil infested with the Fusarium from muskmelon. When muskmelon was used as a stock and muskmelon or cucumber as scions, however, the muskmelon-on-muskmelon developed typical wilt, and the cucumber-on-muskmelon showed typical wilting symptoms in the stock, and the scion wilted and died. Hendrix et al (3) in 1948 made numerous single-spore isolates of Fusarium wilt fungi from muskmelon and watermelon. In cross-inoculation experiments, isolates of the Fusarium from watermelon attacked muskmelon seedlings; however, each was more virulent on its original host. McKeen (6) in 1951, reported similar results in cross-inoculation experiments.

According to Snyder and Hansen (11) the Fusaria which produce vascular wilts are classified as biological forms of *Fusarium oxysporum*, thus the Fusarium from watermelon would be *F. oxysporum* f. *niveum* and that from muskmelon *F. oxysporum* f. *melonis*. The cucumber Fusarium differs from the watermelon and muskmelon forms in pathogenicity and should be considered a new form of *Fusarium oxysporum* (Schl.) Snyd. & Hans.

In tests on cucumber and muskmelon, results with the cucumber isolates here studied were somewhat similar to those obtained by van Koot (14) with a Fusarium from cucumber. Van Koot did not, however, use watermelon in his tests.

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LITERATURE CITED

1. Behr, L. L. 1948. Krankheiten und Schädlinge der Gurke sowie deren Bekämpfung. Ceres [Hamburg] **1** (7-8): 17-20.
2. Cox, C. E. 1944. The response of cantaloupe-cucumber grafts to inoculation with Fusarium bulbigenum var., niveum f. 2. (Abs.) Phytopathology **34**: 991.
3. Hendrix, J. W., E. P. DuCharme, and H. Murakishi. 1948. Pathogenicity of single spore isolates of the Fusarium wilt organisms of muskmelon and watermelon. (Abs.) Phytopathology **38**: 13.
4. Leach, J. G., and T. M. Currence. 1938. Fusarium wilt of muskmelon in Minnesota. Minn. Agr. Exp. Sta. Tech. Bul. 129, 32 p.
5. Leost, F. C. 1948. Plant diseases and cultural operations. Farming in So. Africa **23**: 304-307, 324. (Abs. in Rev. Appl. Mycol. **28**: 185. 1949).
6. McKeen, C. D. 1951. Fusarium wilt of muskmelon and watermelon in southwestern Ontario. (Abs.) Phytopathology **41**: 26.
7. Owen, J. H. 1953. Occurrence of a Fusarium wilt of cucumber in Florida. (Abs.) Phytopathology **43**: 481.
8. Roll-Hansen, F. 1949. Investigation of Fusarium on cultivated plants in Norway. Norges Landbr. Høgskoles Meld. **29**: 257-264.
9. Selby, A. D. 1899. Further studies of cucumber, melon, and tomato diseases, with experiments. Ohio Agr. Exp. Sta. Bul. 105: 217-236.
10. Smith, E. F. 1899. Wilt disease of cotton, watermelon and cowpea. U. S. Dept. Agr., Div. Veg. Path. and Physiol. Bul. 17, 72 p.
11. Snyder, W. C., and H. N. Hansen. 1940. The species concept in Fusarium. Amer. Jour. Bot. **27**: 64-67.
12. Stone, G. E. 1911. Fusarium disease of cucumbers and other plants. Twenty-third Ann. Rept. Mass. Agr. Exp. Sta. Part 2, p. 62-65.
13. Taubenhaus, J. J. 1920. Wilts of the watermelon and related crops. Tex. Agr. Exp. Sta. Bul. 260, 50 p.
14. Van Koot, Y. 1943. Enkele onderzoekingen betreffence de Fusarium-ziekete bij de komkommer. Tijdschr. Over Plantenziekten **49**: 52-72.
15. Weber, Anna. 1924. Tomat- og Agurksygdomme. Gartner-tidende **40**, 16 p. (Abs. in Rev. Appl. Mycol. **4**: 323. 1925.)
16. Weber, G. F. 1925. Diseases of cucumber. Fla. Agr. Exp. Sta. Bul. 177: 27-71.

ANTIBIOSIS IN RELATION TO PINK ROOT OF SHALLOTS[1]

T. E. Freeman and E. C. Tims [2]

SUMMARY

A comparative study was made of 2 soils from the same field, one of which was heavily infested with the pink root fungus while the other was apparently noninfested. The greatest difference between them was in the total number of actinomycetes and in the number of these forms that were antagonistic to the pink root fungus. The noninfested soil contained appreciably more actinomycetes than the infested soil, with a larger proportion of antagonistic forms. Further experiments revealed that specific actinomycetes and other soil microorganisms reduced the severity of the pink root disease. It was concluded that antagonistic actinomycetes might be one of the factors that limited the development of the pink root fungus in the noninfested soil.

In the winter of 1952 a field of shallots in LaFourche Parish, Louisiana, was examined for pink root, a disease caused by *Pyrenochaeta terrestris* (Hansen) Gorenz, J. C. Walker & Larson. In 1 portion of the field the soil was a silty clay type (Mhoon silty clay). The shallots growing in this area showed severe pink root. In an adjacent portion of the field with a heavier clay soil (Sharkey clay), pink root was not observed. According to the landowner, pink root had occurred in 1 area and not in the other for a number of years, even though both areas had been treated similarly and apparently had had equal chances of becoming infested with the pink root fungus. Tims (6) reported in 1953 that once a field had become infested, the disease was likely to become more severe the more often shallots were planted. He stated that there were exceptions, however, and in some cases where shallots were planted year after year the disease apparently became little or no worse. This indicated that some factor or factors might have been keeping the disease in check in some areas.

Various workers (1, 3) have reported that antagonistic soil-borne microorganisms may have a limiting effect on certain diseases caused by soil-borne pathogens. Johnson (4) found that certain soil-inhabiting microorganisms were antagonistic to the pink root fungus when tested in culture. It had previously been reported that antagonistic microorganisms, especially actinomycetes, might be one of the factors limiting pink root development in the heavy clay soil (2).

The object of the present investigation was to determine some of the factors that might account for the failure of pink root to develop in plants in a certain portion of a field while many plants in an adjoining area were diseased. The studies included 1) a comparison of the pH, moisture content, and available nutrients of the 2 soils, 2) a comparison of the total number of actinomycetes occurring in the 2 soil types, 3) a determination of the number of these forms that were antagonistic to the pink root fungus, and 4) a study of the effect of these antagonistic forms on the development of the disease in plants grown in infested soil.

MATERIALS AND METHODS.—Samples of the infested soil (Mhoon silty clay) and noninfested soil (Sharkey clay) were collected in 1953 on May 1, July 11, September 8, and October 10, and in 1954 on January 8 and March 10. Each sample was a composite of several lots taken at random from the area under study.

A method was devised for the screening of large numbers of actinomycetes for ability to suppress the pink root fungus in culture. Two separate layers of media in Petri dishes were used. The bottom layer was Conn's glycerol-asparaginate agar (5), a selective medium for growth of actinomycetes. The second layer consisted of Czapek's sucrose-nitrate agar (5), a suitable medium for growth of *P. terrestris*.

Each soil was diluted 1:200,000 according to the procedure used by Cooper and Chilton (1). One ml of the final dilution was then added to a tube containing approximately 15 ml of Conn's agar. After agitation, the medium was poured into a sterile Petri plate and incubated at 23°C for 4 days, after which the colonies of actinomycetes were counted and their number recorded. Then, 1 ml of a chopped mycelial suspension of *P. terrestris* was added to approximately 10 ml of Czapek's agar and the mixture poured over the plate of Conn's agar on which the actinomycetes were growing. After a 4-day incubation period, the plates were examined for antagonistic actinomycetes. Colonies of such organisms had clear zones around them of varying size depending upon the degree of antagonistic action (Fig. 1).

Suspensions of chopped mycelium were used instead of spore suspensions because of the erratic sporulation of *P. terrestris*. The suspensions were obtained by growing the fungus in liquid Czapek's medium in a flask containing chips of glass. The glass chips, together with 25 ml of medium, were placed in a 250-ml flask and autoclaved. The fungus was then introduced into the flasks and allowed to grow. The flasks were shaken at intervals so that the sharp edges of

[1] Accepted for publication March 22, 1955.

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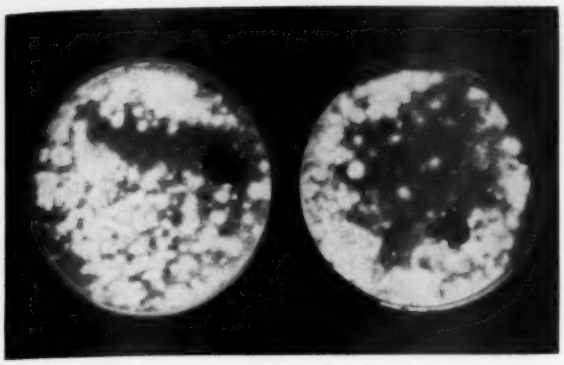

FIG. 1. Typical appearance of actinomycetes showing antagonism toward *P. terrestris* when the dual-agar-layer method is used.

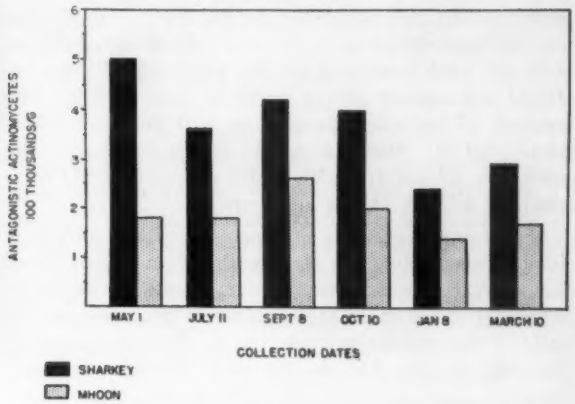

FIG. 2. Average antagonistic actinomycete population in the 2 soils of the area studied on each of 6 collection dates.

the glass chips cut the mycelium into small sections that could be picked up with a sterile pipette.

Three dilutions, consisting of 10 plates for each dilution, were made for each soil on each collection date. The approximate number of antagonistic forms was calculated by multiplying the average number per plate by the dilution factor used, which in all cases was 1:200,000.

Several actinomycetes which had shown antagonism toward the pink root fungus by the method described were tested in sterilized soil artificially infested with the pink root fungus. The inoculum was prepared from 8-day-old cultures of the fungus grown on Czapek's medium in Petri plates. It was ground in a Waring Blendor and mixed with sterile soil in 4-in. pots at the rate of 1 Petri-plate culture per pot. The actinomycetes introduced into the soil were 7-day-old cultures grown on oatmeal agar in Petri plates. The cultures were cut into small sections and mixed thoroughly in the soil at the rate of 1 Petri-plate culture per pot. Both the actinomycetes and the pink root fungus were introduced into the pots at the same time. Since shallots and onions show a similar reaction to pink root, a variety of onions (White Grano) highly susceptible to pink root was used in all greenhouse tests.

EXPERIMENTAL RESULTS.—The pH, moisture content, and available nutrients of the 2 soils did not differ widely. The pH values were slightly higher in the Sharkey soil than in the Mhoon soil; however, there was an overlapping of pH values, i.e., the range for the Mhoon soil was from 5.8 to 6.3 while that of the Sharkey soil was from 6.1 to 6.8. Although the moisture content fluctuated with the prevailing weather conditions at the time of collecting the samples, the moisture content of the Sharkey soil was in all cases slightly higher than that of the Mhoon soil. The available inorganic nutrients in the 2 soils did not differ appreciably, but the Sharkey soil did contain larger amounts of organic matter. The latter soil type was also somewhat lower in available phosphorus and higher in available potassium.

A total of 13,211 actinomycete colonies were tested for ability to suppress the pink root fungus; 7,473 were from the Sharkey soil and 5,738 from the Mhoon soil. A total of 532 actinomycete colonies were found to be antagonistic to the pink root fungus; 355 were from the Sharkey soil and 177 from the Mhoon soil. On each of the 6 dates, the average population of antagonistic actinomycetes was greater in the Sharkey soil than in the Mhoon soil (Fig. 2). The difference ranged from a low of approximately 100,000 per g on January 8, 1954, to a high of approximately 320,000 per g on May 1, 1953. The average difference was 179,000 per g. On July 11, 1953, the actinomycete population was higher in the Mhoon soil than in the Sharkey soil, but the antagonistic population was still higher in the Sharkey soil than in the Mhoon soil (Fig. 2).

Thirty-eight actinomycetes that had shown antago-

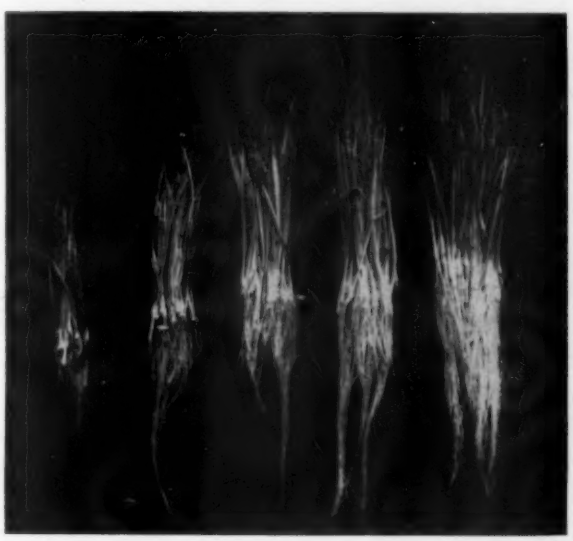

FIG. 3. Varying degrees of antagonism to pink root on onion seedlings shown by different actinomycetes. Non-infected control on extreme right.

nism toward the pink root fungus by the dual-layer method were tested in steamed soil artificially infested with the pink root fungus. All except 4 of them reduced the amount of pink root to some extent. The amount of reduction ranged from 0 to almost complete control. Plants grown in soil infested with the pink root fungus and with actinomycetes isolated from Sharkey soil are shown in Figure 3.

When equal amounts of inoculum of the pink root fungus were added to sterilized and to nonsterilized soil, pink root was more severe in plants grown in the sterilized soil than in those grown in the nonsterilized soil. These results are similar to those reported by Johnson (3) in his studies with *Pythium arrhenomanes.*

DISCUSSION AND CONCLUSIONS.—It was apparent that some factor or factors prevented pink root in plants grown in the Sharkey type soil and that these factors were not operative in the adjacent Mhoon type soil. The fact that both areas had been treated similarly for a number of years indicated that the limiting factor or factors occurred naturally and were not induced by cultivation practices. That differences in populations of antagonistic actinomycetes may be one of the factors involved is suggested by the rather consistent results of this study.

The pH and moisture content of the soil apparently were not important factors in determining the number of actinomycetes present. Since the organic content of the Sharkey soil was higher than that of the Mhoon soil, this is probably the most important factor. Even though the soils were collected so that all 4 seasons were represented, no definite conclusion could be reached from the data as to the seasonal effect on the total antagonistic actinomycete population.

The fact that pink root was more severe in plants grown in sterilized artificially infested soil than in those grown in nonsterilized artificially infested soil indicates that microorganisms in general do in some way tend to suppress pink root.

A larger number of antagonistic actinomycetes were found in the Sharkey soil that was free of the pink root fungus than in the infested Mhoon soil. Certain of these forms reduced the amount of pink root in plants grown in artificially infested soil. These facts seem to indicate that certain actinomycetes may have played some part in limiting the development of pink root in plants grown in the Sharkey clay soil of the field under observation.

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LITERATURE CITED

1. COOPER, W. E., AND S. J. P. CHILTON. 1950. Studies on antibiotic soil organisms. I. Actinomycetes antibiotic to Pythium arrhenomanes in sugarcane soils of Louisiana. Phytopathology **40**:544-552.
2. FREEMAN, T. E., AND E. C. TIMS. 1954. Antibiosis as a possible limiting factor of pink root of shallots. (Abs.) Phytopathology **44**: 488.
3. JOHNSON, L. F. 1952. The relation of antagonistic microorganisms to pythium root rot of sugarcane and corn in recontaminated soils. La. Acad. Sci. Proc. **15**: 24-31.
4. JOHNSON, L. F. 1953. The effect of antagonistic soil microorganisms on the severity of pythium root rot of sugarcane. Unpubl. Dissertation. Louisiana State University.
5. LEVINE, M., AND H. W. SCHOELEIN. 1930. A compilation of culture media for the cultivation of microorganisms. Williams and Wilkins, Baltimore. 969 p.
6. TIMS, E. C. 1953. Pink root of shallots, Allium ascalonicum. U. S. Dept. Agr. Pl. Dis. Rptr. **37**: 533-537.

PRELIMINARY SYMPOSIA AT THE ATLANTA MEETING SPONSORED JOINTLY BY AAAS AND APS TUESDAY, DECEMBER 27, 1955

9:00 a.m. ***The Tobacco Plant. Municipal Auditorium.***
W. G. FRANKENBURG, General Cigar Co. Chemical Changes in Compounds of Tobacco Leaf and Cigar Smoke.
M. L. ZUCKER, Conn. Agr. Exp. Sta. Light and the Structure of the Tobacco Leaf.
H. H. SMITH, Cornell University. Tobacco Genetics.
E. E. CLAYTON, American Sumatra Corp. Tobacco Pathology.

1:30 p.m. ***The Cotton Plant. Municipal Auditorium.***
SPEAKER TO BE DETERMINED. Cotton Biochemistry.
WANDA K. FARR, St. Clairsville, Ohio. The Cotton Fiber.
S. G. STEPHENS. N. C. State College. Cytogenetics of Cotton in Relation to Breeding Problems.
J. T. PRESLEY, U. S. Dept. Agr. Cotton Pathology.

EFFECT OF NUTRIENT SPRAYS ON FUSARIUM WILT OF TOMATO [1]

J. R. Bloom and J. C. Walker

SUMMARY

Various nutrient elements were applied to tomato plants as foliage-spray supplements to a balanced solution supplied through the roots. When nitrogen in the form of urea was applied before inoculation of the plants with *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyd. & Hans., the disease was retarded progressively with increasing concentrations; when it was applied after inoculation, disease development increased with increase in concentration. When potassium chloride was applied as a foliage nutrient before inoculation, no significant effect on wilt development was observed. When it was applied after inoculation, there was an increase in disease index with increase in concentration of the nutrient. When sodium chloride was used, the effects were similar to those with potassium chloride. Calcium chloride, on the other hand, had no significant influence on wilt. When potassium chloride and calcium chloride were combined in the foliage spray, the effects of the former were counteracted. The effects of monobasic sodium phosphate, sodium glycerophosphate, magnesium sulfate, and magnesium chloride were not significant.

In a study of wilt (incited by *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyd. & Hans.) in tomato seedlings grown in quartz sand culture, Walker and Foster[2] showed that development of the disease was enhanced when a low concentration of potassium or a high concentration of nitrogen was used in the mineral nutrient supplied the host. A high concentration of potassium or a low concentration of nitrogen had an opposite effect. In a later study, Foster and Walker[3] reported on the influence of high and low levels of potassium and nitrogen when supplied to seedlings for 30 days before inoculation and with a balanced nutrient after inoculation. In the latter study, the effects were the reverse of those obtained when high or low levels were used throughout the experiments. The disease developed most rapidly in plants supplied before inoculation with low-nitrogen or high-potassium solutions and least rapidly when supplied with high-nitrogen or low-potassium solutions. The same effects were found with Bonny Best, a very susceptible variety, and with Marglobe, a partially resistant variety. The differences between the effects of the high and low concentrations of the respective elements were greatest, however, with the Marglobe variety. In the experiments cited, all nutrients were applied through the roots. The present paper is a report on a study of the influence on the disease of supplementary foliage sprays containing various concentrations of several elements.

MATERIALS AND METHODS.—Bonny Best, a wilt-susceptible variety of tomato, was used. Seed was sown in quartz sand and watered with distilled water. Two weeks later seedlings were transplanted to quartz sand in 8-in. pots, 10 plants to a pot. The pots were set up in drip culture and watered with 1H Hoagland's solution as described by Gallegly and Walker.[4] The greenhouse temperature was kept at about 28°C. The wilt fungus was grown on Richard's solution for 2 weeks. The mycelium and spores were then removed from the culture and washed with distilled water by means of a Buchner funnel. The fungus mat was suspended in distilled water and ground in a Waring Blendor. Plants were inoculated when about 6 in. tall (about 30 days after transplanting) by removing them from the sand, immersing the roots in the fungus suspension, and replanting in the original pots.

To avoid foliage injury, various concentrations of materials to be used as sprays were made up in 0.7 M sucrose. When applications were made up to the time of inoculation, they were begun 1 week after transplanting, were continued at weekly intervals up to inoculation, and were then discontinued. When they were made only after inoculation, they were begun after 1 week and continued at weekly intervals until the end of the experiment. On successive days between treatments and for several days after the last treatment, the foliage was sprayed with 0.1 per cent solution of detergent (Vel[5]) in distilled water to redistribute the residue which remained on the surface.

Symptoms usually appeared at about 10 days after inoculation. At the end of each experiment, plants in each treatment were placed in 1 of 5 disease classes. A disease index was calculated according to the method of Gallegly and Walker.[4] Each experiment consisted of 4 randomized replicate blocks with 40 plants in each treatment replicate. There was usually a period of about 30 days from the original transplant-

[1] Accepted for publication March 25, 1955.

This investigation was supported by a grant from the Research Committee of the Graduate School, University of Wisconsin.

[2] Walker, J. C., and R. E. Foster. 1946. Plant nutrition in relation to disease development. III. Fusarium wilt of tomato. Amer. Jour. Bot. 33: 259-264.

[3] Foster, R. E., and J. C. Walker. 1947. Predisposition of tomato to Fusarium wilt. Jour. Agr. Res. 74: 165-185.

[4] Gallegly, M. E., Jr., and J. C. Walker. 1949. Plant nutrition in relation to disease development. V. Bacterial wilt of tomato. Amer. Jour. Bot. 36: 613-623.

[5] Manufactured by Colgate-Palmolive-Peet Co., Jersey City, N. J.

TABLE 1.—*Indices of the development of Fusarium wilt in tomato plants sprayed with urea before and after inoculation*

Molar conc.	Disease indices[a]	
	Sprayed before inoculation	Sprayed after inoculation
0	60.0	56.0
0.1	61.5	52.5
0.2	65.5	62.5
0.3	49.0	69.5
0.4	48.0	70.5
LSD(19:1)	13.5	7.5

[a] Calculated from a scale in which 0 indicates no symptoms and 100 represents severe wilt. Each figure is the average for 160 plants.

TABLE 2.—*Indices of the development of Fusarium wilt in tomato plants sprayed with potassium chloride before and after inoculation*

Molar conc.	Disease indices[a]	
	Sprayed before inoculation	Sprayed after inoculation
0	62.0	47.5
0.12	67.0	48.5
0.24	62.5	62.5
0.36	64.0	64.0
0.48	62.5	74.0
LSD (19:1)	NS	13.3

[a] Calculated from a scale in which 0 represents no symptoms and 100 represents severe wilt. Each figure is the average for 160 plants.

ing to the time of inoculation and a period of about 40 days from inoculation to the final disease reading.

RESULTS.—*Effect of nitrogen.*—Urea was used as a source of nitrogen in the foliage spray. It was applied at 0.1, 0.2, 0.3, and 0.4 M concentrations. The results of a typical experiment are presented in Table 1. There was a progressive decline in disease index with increase in concentration of urea when applications were by foliage spray before inoculation. On the contrary, there was a progressive increase in disease index with increase in concentration when urea was applied as a foliage spray after inoculation. The application of nitrogen through the foliage before inoculation tended to render plants less favorably disposed to disease development, as Foster and Walker[3] found to be the case when high-nitrogen nutrient was applied through the roots up to the time of inoculation. The application of nitrogen through the foliage after inoculation had the same effect as when it was supplied through the roots before and after inoculation.[2]

Effects of potassium, sodium, magnesium, and calcium.—Potassium was applied as potassium chloride at 0.12, 0.24, 0.36, and 0.48 M concentrations. The results of a typical experiment are presented in Table 2. No significant differences were obtained when the spray was applied before inoculation. When the spray was applied after inoculation, there was a progressive increase in disease index with increase in concentration of the nutrient. Magnesium sulfate and magnesium chloride applied before inoculation tended to reduce the disease index, but differences among treatments were not significant; when they were applied after inoculation no appreciable effects were noted. When calcium chloride was applied at 0.07, 0.14, 0.21, 0.28 M concentrations before and after inoculation, there were no significant differences among the effects of treatments, indicating that the Cl ion had little or no effect. When potassium chloride and calcium chloride were applied together at molar concentrations of 0.12 and 0.08, 0.24 and 0.16, 0.36 and 0.24, 0.48 and 0.32 M, respectively, there were no significant differences among treatments, indicating that the Ca ion counteracted the effect of the K ion. Although the application of high potassium nutrient through the roots before inoculation was found by Foster and Walker[3] to render the plants more favorably disposed to wilt, application of potassium to the foliage spray before inoculation had no appreciable effect. After inoculation, however, increasing amounts of potassium applied in the foliage spray increased disease development, whereas Walker and Foster[2] found that high-potassium nutrient supplied through the roots before and after inoculation retarded the progress of the disease.

Effects of phosphorus.—When monobasic sodium phosphate or sodium glycerophosphate was applied in various concentrations as foliage sprays before and after inoculation, no consistent effects on wilt were observed.

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IMMUNITY TO VIRUS X IN POTATO: STUDIES OF SEGREGATING SEEDLING POPULATIONS [1]

Roland G. Timian,[2] W. J. Hooker,[3] and C. E. Peterson [4]

SUMMARY

Mechanical inoculation of small potato seedlings with suitable isolates of virus X is a reliable means of identifying susceptible segregates. Local symptoms developed at 16°C within a week and systemic symptoms within 2 weeks in susceptible seedling transplants when certain isolates of virus X were used for inoculation. Seedling plants immune to virus X failed to develop either local necrosis or systemic mottle following inoculation and the virus was not carried systemically in such plants.

Marked differences in the number of plants with symptoms were obtained with different isolates of virus X. One isolate, X5, was very effective in inciting both local and systemic infection. It was the only one that incited visible symptoms in all inoculated plants of a susceptible progeny. Another isolate, XRS, was approximately as effective in inciting local symptoms, but was relatively ineffective in inciting systemic symptoms. One isolate, X8, was very mild and incited symptoms in only a small number of seedling plants. The other isolates tested were intermediate between these 2 extremes.

In tests with 2 isolates inciting severe symptoms, X5 and XRS, local symptoms developed equally well at 18°C and at 24°, whereas systemic symptoms were more pronounced at 24° than at 18°. At 10°, symptoms incited by isolate X5 were slower to develop than at either of the higher temperatures and no plants inoculated with XRS developed systemic symptoms.

In the greenhouse, the number of seedling plants that developed symptoms was increased when light intensity was reduced by shading.

In the potato improvement program of the Iowa Agricultural Experiment Station, the marked resistance or immunity of S 41956 (6) is used as a source of resistance to virus X. Literature concerning the different types of resistance to virus X in potato was recently reviewed (3) and the advantages of using the immunity of S 41956 in a breeding program were pointed out.

Practically no research has been reported on factors influencing infection by virus X and symptom development in small seedling potato plants following mechanical inoculation. A more complete understanding of these factors is desirable for successful identification of susceptible seedlings in a potato breeding program. In an earlier paper, Timian, Hooker, and Peterson (9) reported that clonal lines of potato susceptible to virus X as determined by the graft test of Raleigh (5) developed either local or systemic symptoms when mechanically inoculated with certain isolates of the virus, whereas immune lines did not exhibit symptoms and were not symptomless carriers following mechanical inoculation. The mechanical test for immunity to virus X was believed to be equal or superior in reliability to the graft test of Raleigh (5) and had the advantage of greater ease of testing.

In order to adapt such a mechanical inoculation test to a program of breeding potatoes for immunity to virus X, further studies were made on populations of seedling potatoes before they were transplanted to the greenhouse bench. This paper, of which a preliminary report has been published (8), describes investigations on small seedlings of potato from a number of progenies grown under various environmental conditions and inoculated with different isolates of virus X.

MATERIALS AND METHODS.—Virus X isolates described previously (9) and an additional one, XRS, were maintained in plants of *Nicotiana glutinosa* L. under insect-proof cages in the greenhouse. XRS was a severe ring spot isolate of virus X obtained from R. H. Larson of the Department of Plant Pathology, University of Wisconsin. Sap from plants infected with virus X was diluted 1–10 with water and used with carborundum (400-mesh) in mechanical inoculation tests.

Potato plants were inoculated individually in the seedling flats when the second true leaf was 3–6 mm wide. In a few tests inoculation was delayed until after transplanting when plants were in the 5–6 leaf stage. Inoculum and abrasive were rubbed gently on the leaves with the thumb and forefinger, and plants

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were washed with a fine spray of water after inoculation.

Potato progenies and their parents used in these tests were as follows:

Ia 940 (B792-88 × Cherokee), immune × susceptible;
Ia 941 (B792-94 × Cherokee), immune × susceptible;
Ia 943 (S 41956 × Cherokee), immune × susceptible;
Ia 1236 (ND457-1 selfed), susceptible;
Ia 1237 (Ia 940-1 selfed), susceptible;
Ia 1238 (B792-94 selfed), immune; and
Ia 1239 (Ia 902-4 selfed), immune.

Progeny numbers beginning with Ia are from the potato breeding program of the Iowa Agricultural Experiment Station and the U. S. Department of Agriculture, B and S are from the U. S. Department of Agriculture and ND from the North Dakota Agricultural Experiment Station.

EXPERIMENTAL RESULTS.—*Symptoms in potato seedlings infected with virus X.*—Seedling potato plants of 2 progenies segregating for immunity to virus X, Ia 941 and Ia 943, were inoculated mechanically with isolate XRS at the 6-leaf stage. The 2 youngest fully expanded leaves were marked with India ink and rubbed with an inoculum-carborundum mixture. These plants were rubbed a second time 2 weeks later. No additional individuals developed symptoms following the second inoculation, indicating that no susceptible plants previously had escaped infection. Greenhouse temperatures averaged 17°C with extremes of 13° and 33°.

After inoculation, susceptible plants developed local necrotic symptoms (Fig. 1, A) within 5–11 days and systemic symptoms (Fig. 1, B) within 10–15 days (Table 1).

Leaves of the inoculated seedling plants were tested on plants of *Gomphrena globosa* L. for the presence of virus X. Plants with systemic symptoms were infected with virus X, whereas those remaining symptomless throughout the period of observation were free of the virus. Of those with only local symptoms none was systemically infected. After harvest, tubers from these plants were tested on plants of *G. globosa* by the method of Hoyman (4). Results were identical to those of the first test except that 1 plant which was virus free according to the first test produced infected tubers. Tubers of all plants in this test were planted in the field for increase so that further tests could be made. Of special interest were those plants with local symptoms and no systemic infection. This planting was later lost in a flood.

The trial demonstrated that small seedling plants in the 6-leaf stage could be tested for immunity to virus X and that those with neither local lesions nor systemic mottle were immune to the virus. It was later determined that isolate XRS was very effective in inciting local symptoms but that it was relatively inferior to other isolates in establishing systemic infection. Judging from subsequent observations, it seems probable that most plants that produced only local lesions in this test would have become invaded systemically had they been inoculated with certain other isolates of virus X.

TABLE 1.—*Percentage of seedling potato plants with symptoms at intervals following inoculation of a population segregating for immunity to virus X*

Progeny	Time after inoculation	Symptoms: none	Symptoms: local only	Symptoms: local and systemic
	days	%	%	%
Ia 941[a]	6	63	37	0
	11	52	27	21
	27	52	9	39
Ia 943[b]	6	66	34	0
	11	48	45	7
	27	48	21	31

[a] 128 plants inoculated with isolate XRS.
[b] 126 plants inoculated with isolate XRS.

Effectiveness of different isolates of virus X in the identification of susceptible seedling plants.—Ten virus X isolates previously described (9) were compared for effectiveness in the identification of susceptible seedling plants, and data from 6 isolates are presented (Fig. 2). For this, 2 progenies uniformly susceptible to virus X (progenies Ia 1236 and Ia 1237, designated in Figure 2 as S 1 and S 2, respectively) were compared with 2 progenies segregating for immunity to virus X (Ia 1238 and Ia 1239, designated as SI 1 and SI 2, respectively). Seedling plants were inoculated at an early age and held in the greenhouse at 16°C. Data (Fig. 2) are derived from reactions of approximately 90 plants of each progeny with 30 plants per treatment in each of 3 replications. The percentages of plants with local and with systemic symptoms were determined for each isolate and for each progeny at intervals from the fifth day after inoculation until the twentieth day.

Local symptoms on potato seedlings consisted of punctate necrotic spots which enlarged and often involved the entire leaf. These were similar in appearance for all of the isolates tested. Affected leaves usually abscised in 10–20 days (Fig. 1, C). Systemic symptoms (Fig. 1, D, E) were necrotic streaks following the small veins in the leaves, and only occasionally did systemic mottle develop on these small plants.

Isolate X5 was superior to the other isolates tested in inciting both local and systemic symptoms in all 4 progenies. It was the only one that infected all inoculated plants of the susceptible progeny. Isolate XRS was almost as effective as X5 and was superior to the other isolates in inciting local lesions. It was not as effective as X5 or X7 in inciting systemic symptoms. The percentages of plants that developed symptoms following inoculation with X4 or X8 were so low that these isolates were relatively ineffective in identifying susceptible plants. Isolates ranked

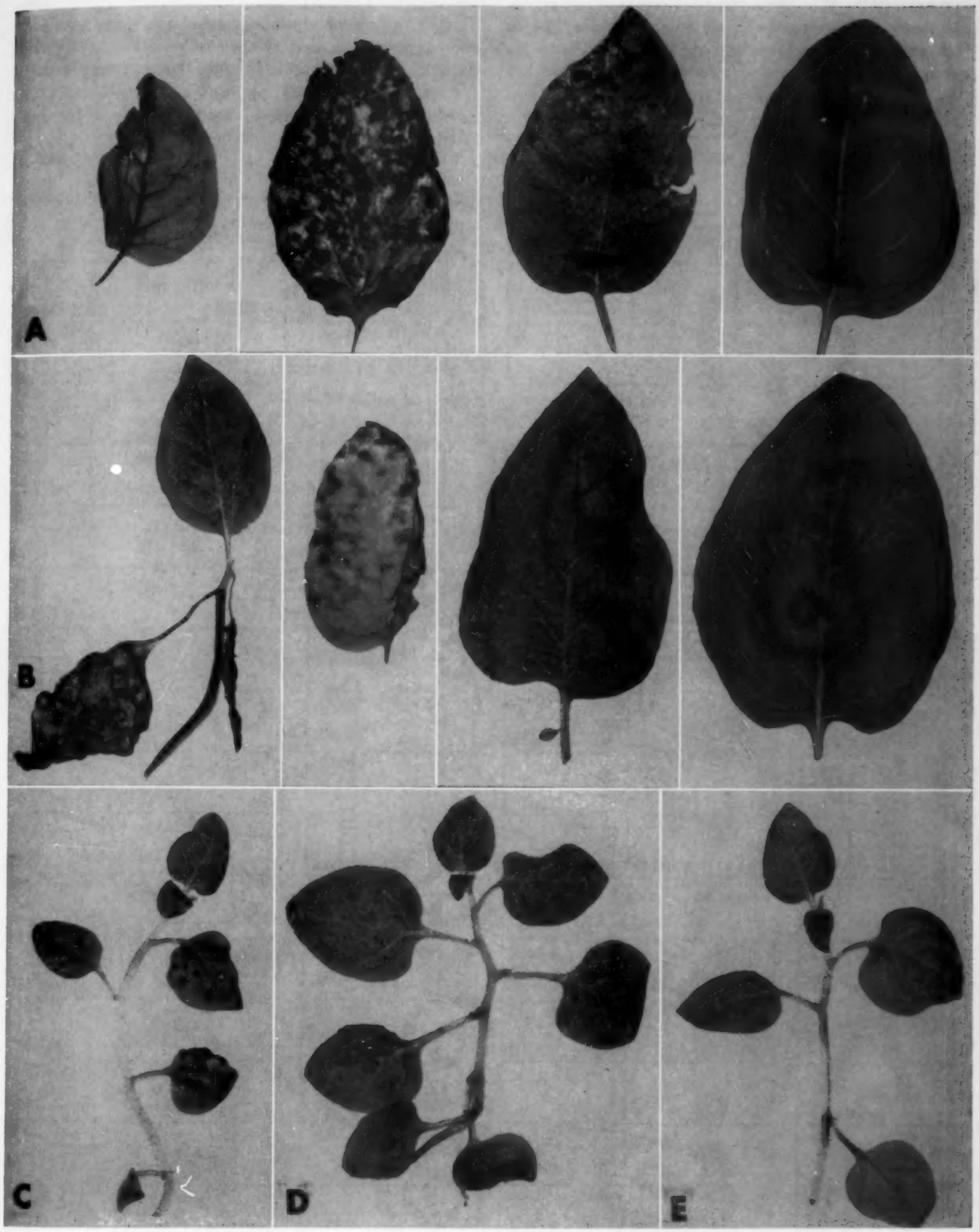

FIG. 1. A, B). Typical symptoms on leaves of seedling potato transplants following inoculation with virus X; A, local lesions on inoculated leaves; and B, systemic symptoms. The symptomless leaf at the right in A and that in B are typical of plants immune to virus X. C, D). Symptoms on seedlings before transplanting following inoculation with certain isolates of virus X; C, local lesions; D, systemic necrosis; and E. systemic stem necrosis. Photographs by R. E. Wicklund.

approximately the same for earliness of symptom expression on potato seedlings as for percentages of plants expressing symptoms. Similar results were obtained in a less extensive earlier trial.

Influence of temperature on symptom development.—Plants of a susceptible progeny, Ia 1236, were inoculated with X5 and XRS in greenhouses at 10°, 18°, and 24°C. Approximately 60 plants were inoculated at each temperature and symptoms were recorded from the time of inoculation through the seventeenth day.

Local symptoms following inoculation with X5 developed at approximately the same rate at 18° and

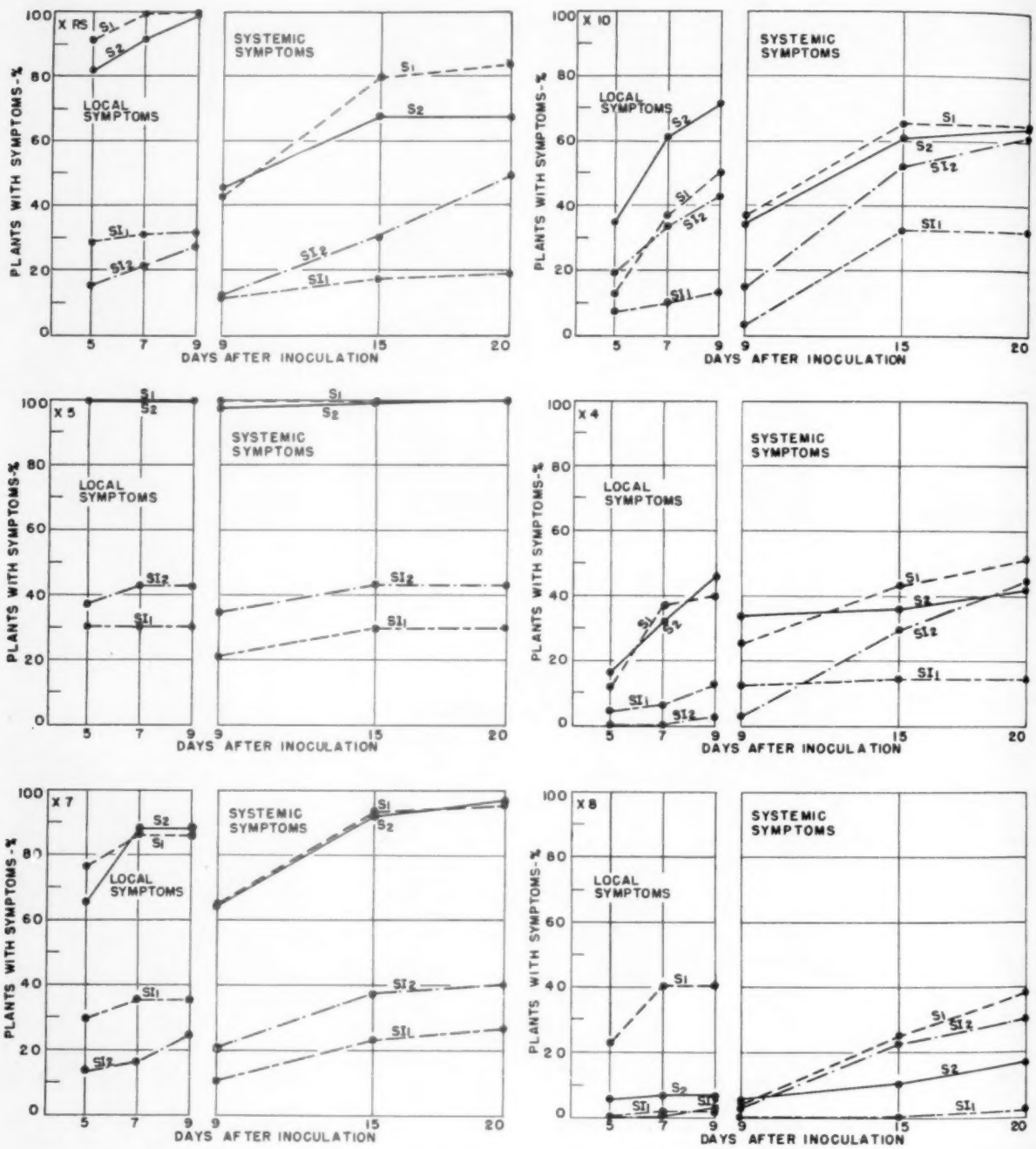

FIG. 2. Percentage of seedling potato plants with symptoms in 4 progenies inoculated with 6 isolates of virus X. Progenies S 1 and S 2 were uniformly susceptible and progenies SI 1 and SI 2 were segregating for immunity to virus X.

24° (Fig. 3). At 10° there was a 3-day delay. Systemic symptoms developed most rapidly at 24°, were somewhat slower to develop at 18°, and were delayed approximately 1 week at 10°C. The number of plants ultimately developing either local or systemic symptoms were essentially the same at all temperatures.

Isolate XRS was effective in inciting local symptoms at each temperature although there was a delay of somewhat more than a week at 10°C. There were no plants with systemic symptoms at 10°. Less than 30 per cent of the plants developed systemic symptoms at 24°, and a slightly smaller number showed symptoms at 18°.

In another test with a progeny segregating for immunity to virus X, Ia 1238, symptom expression in susceptible plants was similar to that in the susceptible progeny. Most of the plants with local symptoms following inoculation with isolate X5 at 18°C and 24° later expressed systemic symptoms. Although the number of susceptible plants in this segregating population was small, results were in agreement with the previous test involving a completely susceptible progeny.

Influence of light intensity on symptom expression.—To determine the influence of light intensity and temperature on symptom development, progeny Ia 940, segregating for immunity to virus X, was grown in a split-plot design in which the smallest plot consisted of approximately 50 plants. The main-plot treatments were 2 temperatures, 16°C and 28°, and the sub-plot treatments were 2 light intensities. Plants were grown in winter in the greenhouses. Those at the low light intensity were shaded with a double layer of cheesecloth and received approximately 50 per cent of the light received by the unshaded plots. Light intensities between 11:00 a.m. and 2:00 p.m. on bright days were 953 and 537 ft.-candles in unshaded and shaded plots, respectively. Light intensities on cloudy days were 384 and 210; on very dark days, 31 and 12; and averaged 634 and 334, respectively. Final readings were made 16 days after inoculation with isolate XRS. The percentages of infected plants (Table 2) were converted to angles by the arc-sine transformation (7) and an analysis of variance was calculated. The percentage of plants with symptoms was significantly greater at the low temperature than at the

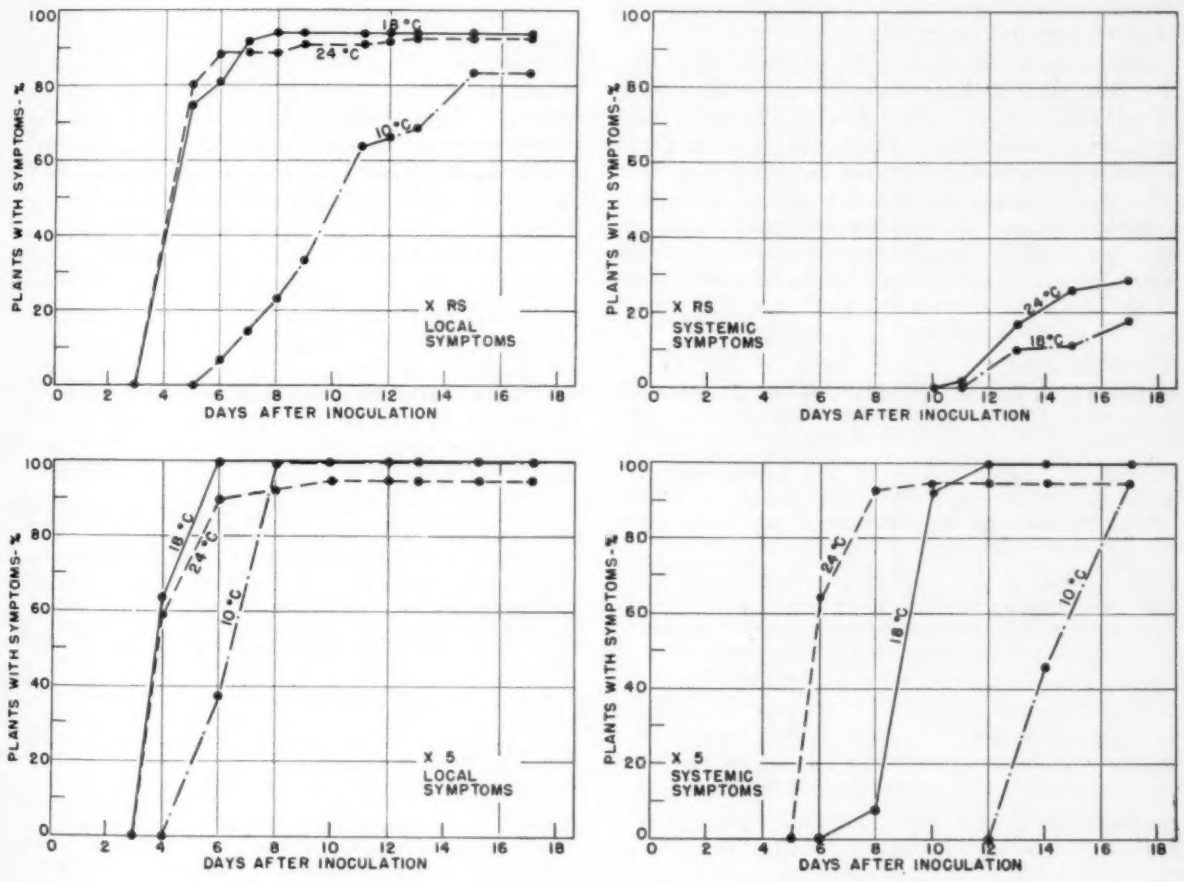

FIG. 3. Percentage of plants with local and systemic symptoms in a susceptible progeny of potato seedlings at 3 temperatures following inoculation with X5 and XRS.

TABLE 2.—*Percentage of plants with symptoms in a population of potato seedlings segregating for immunity to virus X following inoculation with isolate XRS at 2 temperatures and 2 light intensities*

Temperature	Light intensity	Plants inoculated	Plants with symptoms
		no.	%
16°C	reduced	182	74.7
	high	191	67.5
	average	186	71.1
28°C	reduced	210	44.3
	high	188	30.3
	average	199	37.3

high temperature and was also significantly greater at the low than at the high light intensity. In a second experiment, involving 2 progenies at 2 light intensities, differences in disease incidence were similar to those in the first experiment and again were significant.

DISCUSSION.—The mechanical inoculation test for identification of potato plants immune to virus X has been used successfully for identification of potato plants maintained as clonal lines (9). The test can also be applied successfully to small seedling potato plants before transplanting.

Symptoms in seedling potato plants following infection with virus X were similar to those observed in clonal lines (9). In general, symptoms were more severe in small seedling plants than in the larger plants grown from tubers. Symptoms in the former were very similar to top necrosis, a symptom common to certain varieties hypersensitive to virus X. Isolates of Virus X, when compared in ability to incite symptoms, ranked essentially the same in either small seedlings or in plants grown from tubers. When seedlings of susceptible progenies were inoculated with certain isolates of virus X, all individuals became infected. Individuals immune to virus X did not evidence infection either as plants grown from tubers (9) or as small plants developed from true seed.

The percentage of susceptible plants with symptoms was influenced greatly by the isolate of virus X used for inoculation. Thus, for identification of immune plants in the breeding program, isolates that incited severe symptoms were more effective than those inciting mild symptoms. One isolate, X5, incited visible symptoms in all individuals of a susceptible progeny. The ability of this isolate to incite systemic symptoms in a high percentage of the susceptible individuals is a distinct advantage in the breeding program because systemic symptoms are more easily recognized in a population of small seedlings than are local symptoms.

The temperature at which inoculated plants were held did not greatly affect the number of plants that eventually showed symptoms. Symptoms were evident over the temperature range of 10–24° suggesting that precise greenhouse temperatures would not be required for identification of susceptible seedlings. Since symptoms were accentuated and the number of plants infected was increased by shading of seedling plants following inoculation, it is probable that intense light should be avoided in routine selection of resistant seedlings. Increased severity of symptoms and increased susceptibility to infection in plants subjected to reduced light has been reported with potato virus X (1) and with certain other viruses (2).

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LITERATURE CITED

1. BAWDEN, F. C. 1950. Plant viruses and virus diseases. Ed. 3. Chronica Botanica Co. Waltham, Mass. 335 p.
2. BAWDEN, F. C., AND F. M. ROBERTS. 1947. The influence of light intensity on the susceptibility of plants to certain viruses. Ann. Appl. Biol. **34**: 286-296.
3. HOOKER, W. J., C. E. PETERSON, AND R. G. TIMIAN. 1954. Virus X resistance in potato. Amer. Potato Jour. **31**: 199-212.
4. HOYMAN, W. G. 1951. A method of indexing potatoes for virus X by use of petiole or tuber juice. Amer. Potato Jour. **28**: 713-721.
5. RALEIGH, W. P. 1936. An abnormal graft reaction in potato resulting from virus infection of a scion on a resistant stock. Phytopathology **26**: 795-796.
6. SCHULTZ, E. S., C. F. CLARK, R. BONDE, W. P. RALEIGH, AND F. J. STEVENSON. 1934. Resistance of potato to mosaic and other virus diseases. Phytopathology **24**: 116-132.
7. SNEDECOR, G. W. 1946. Statistical methods. Ed. 4. Collegiate Press, Ames, Iowa. 485 p.
8. TIMIAN, R. G. 1953. Identification of seedling potatoes immune to virus X (Abs.) Phytopathology **43**: 487.
9. TIMIAN, R. G., W. J. HOOKER, AND C. E. PETERSON. 1955. Immunity to virus X in potato: Studies of clonal lines. Phytopathology **45**: 313-319.

TWO NEW CUCUMBER VARIETIES RESISTANT TO SCAB AND MOSAIC [1]

J. C. Walker and C. F. Pierson

SUMMARY

Two new pickling cucumber varieties were released to the trade in 1954 under the names Wisconsin SMR 9 and Wisconsin SMR 12. Both have been shown to be highly resistant to scab and to common cucumber mosaic under field conditions where these 2 diseases were very severe on susceptible varieties. The scab resistant parent was from an F_3 homozygous-scab-resistant progeny from a cross between scab-resistant Maine No. 2 and Chicago Pickling. This was crossed and backcrossed 5 times to M-20, a mosaic-resistant line received from J. D. Wilson of the Ohio Experiment Station. The new varieties resemble National Pickling in vine type and season. The fruits are slightly lighter green in color than National Pickling, Wisconsin SR 6, and Ohio MR 17. Wisconsin SMR 9 has fruits that resemble closely those of Ohio MR 17 in length/diameter ratio. The fruits of Wisconsin SMR 12 are intermediate between National Pickling and Ohio MR 17 in this respect. Under Wisconsin conditions both varieties yield as well as susceptible pickling varieties in the absence of scab and mosaic and outyield the older varieties susceptible to mosaic where the latter is severe.

In a previous paper[2] the development of 2 varieties of pickling cucumber resistant to scab (incited by *Cladosporium cucumerinum* Ell. & Arth.) was reported. One of these, Wisconsin SR 6, is now widely used in Wisconsin and other northern states where scab is a major disease. These varieties are, however, susceptible to common cucumber mosaic, which is also destructive in many pickle-growing areas. Several satisfactorily mosaic-resistant varieties, e.g. Yorkstate Pickling,[3] Ohio MR 17,[4] and Ohio MR 25[5] are available, but each of these is susceptible to scab. The present paper is a report upon the development of 2 new pickling varieties, Wisconsin SMR 9 and Wisconsin SMR 12, which are resistant to both scab and mosaic. These varieties were released in 1954 and seed became available to the trade for use in 1955.

BREEDING PROCEDURE.—The scab resistant parent was from an F_3 homozygous-scab-resistant progeny from a cross between scab-resistant Maine No. 2 and Chicago Pickling. The scab resistant parent was crossed with a mosaic-resistant line, M-20, secured from J. D. Wilson of the Ohio Experiment Station. This line was derived from a cross made by Dr. H. M. Munger of Cornell University between Chinese Long and National Pickling which was backcrossed by him twice to National Pickling. Line M-20 was close to Ohio MR 17 in type but in our trials it was found to be slightly better adapted to Wisconsin conditions and slightly more resistant to mosaic than Ohio MR 17. Beginning with the F_1 progeny, 5 successive backcrosses to M 20 were made. Each backcross progeny was inoculated with the scab fungus in the seedling stage at 17°C as described previously,[6] and the susceptible individuals were discarded. Resistant individuals of the fifth backcross were selfed and the progenies inoculated. Resistant individuals were again selfed and progenies inoculated. Several homozygous-scab-resistant progenies were then increased and subjected to mosaic tests and field evaluation as to type and yield.

EVALUATION AND RESISTANCE TESTS.—In 1953, 4 scab-resistant progenies designated as 12-3, 12-9, 12-12, and 12-17 were planted in the field along with National Pickling (mosaic-susceptible, scab-susceptible), and Ohio MR 17 (mosaic-resistant, scab-susceptible) at Madison, Wisconsin, where scab does not usually occur. Plantings were in 3 randomized blocks of 25-ft. rows. When the plants were in the 3-leaf stage, they were inoculated by sprinkling the first leaf with carborundum and rubbing with an extract of leaves of *Nicotiana glutinosa* L. infected with cucumber virus 1. Under field conditions at Madison mosaic usually appears in susceptible varieties as mottle of the youngest leaves about 4 weeks after inoculation. Symptoms on successive leaves gradually become more severe and suppression of elongation of vines increases gradually. The degree of growth suppression varies from season to season. Mottled fruits increase in number as the season advances. Occasional young leaves of resistant varieties show mild mottle as soon as mottle appears on susceptible plants, but such leaves usually outgrow visible symptoms and suppression of growth is negligible. Occasional very mildly mottled fruits appear on resistant varieties.

Fruits were picked 3 times weekly beginning on July 15, and the percentages of fruits showing any evidence of mosaic were recorded at each picking. The

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This investigation was supported by a grant from the Wisconsin Pickle Packers' Association.

[2] Walker, J. C., C. F. Pierson, and A. B. Wiles. 1953. Two new scab-resistant cucumber varieties. Phytopathology 43: 215-217.

[3] Munger, H. M. 1950. Two new mosaic-resistant cucumbers. Farm Res. (N. Y. State Sta.) 10(2): 20.

[4] Wilson, J. D. 1951. Ohio MR 17. A new mosaic tolerant pickling cucumber. Ohio Agr. Exp. Sta. Res. Cir. 10.

[5] Wilson, J. D., C. A. John, and F. Myrice. 1954. Ohio MR 25. A pickling cucumber highly tolerant to mosaic. Ohio Agr. Exp. Sta. Res. Cir. 25.

[6] Walker, J. C. 1950. Environment and host resistance in relation to cucumber scab. Phytopathology 40: 1094-1102.

TABLE 1.—*Occurrence of mosaic in fruits of inoculated plants of breeding progenies and of standard resistant and susceptible varieties at Madison, Wisconsin, 1953*

Variety or progeny	Percentage of mottled fruits on date indicated[a]					
	Aug. 10	Aug. 12	Aug. 17	Aug. 21	Aug. 24	Aug. 28
National Pickling	13	16	20	64	67	90
Ohio MR 17	5	6	1	1	1	0
12-3	0	0	4	6	0	0
12-9	0	5	2	0	0	0
12-12	0	2	2	13	0	0
12-17	4	5	0	0	0	5

[a] Percentages are based on a total of 75 fruits. Plants were inoculated with cucumber virus 1.

first 3 pickings showed no more than negligible amounts of mosaic in any of the lots. In National Pickling, there was a steady increase in incidence of mosaic up to 90 per cent on August 28 (Table 1). The number of mottled fruits in Ohio MR 17 was negligible throughout the period. Breeding progenies were not significantly different from Ohio MR 17 in amount of disease, showing that sufficient backcrossing had been made to provide acceptable mosaic resistance.

Breeding progenies were planted also in the same year in randomized replicated plots in an isolated location at Madison, Wisconsin, where mosaic did not occur. National Pickling, Ohio MR 17, and Wisconsin SR 6 were included to determine yield potential. The first harvest was on July 15, and cumulative yields of ungraded fruits were determined 4, 5, and 6 weeks after the first picking (Table 2). Progeny 12-12 had significantly greater yield at each time interval than National Pickling, Ohio MR 17, and progeny 12-17. It was higher in yield but not significantly higher than the other 2 progenies and Wisconsin SR 6.

The length-diameter ratio of fruits is an important consideration in the evaluation of varieties for processing. Therefore at 4 pickings during the season 25 fruits taken at random from the harvest were measured (Table 3). National Pickling and Wisconsin SR 6 were consistently the shortest in relation to diameter while Ohio MR 17 was always the longest.

TABLE 2.—*Comparative yields of breeding progenies and standard varieties at Madison, Wisconsin, 1953, in a mosaic-free area*

Variety or progeny	Cumulative yield (bushels/acre) at[a]		
	4 weeks	5 weeks	6 weeks
National Pickling	204	332	442
Ohio MR 17	217	302	392
Wisconsin SR 6	258	384	486
12-3	253	380	478
12-9	250	393	506
12-12	286	442	550
12-17	232	349	445
LSD (19:1)	48	64	78

[a] Intervals following the first picking. Plots were 50-ft. rows in 3 randomized blocks.

TABLE 3.—*Length/diameter ratios of fruits of breeding progenies of standard varieties grown in mosaic-free area*

Variety or progeny	Ratios at picking date indicated[a]				
	Aug. 10	Aug. 17	Aug. 21	Aug. 24	Ave.
National Pickling	2.89	2.90	2.87	2.82	2.87
Ohio MR 17	3.22	3.20	3.09	3.11	3.15
Wisconsin SR 6	2.77	2.83	2.85	2.77	2.80
12-3	3.00	2.98	3.06	3.03	3.01
12-9	3.14	3.13	3.11	3.05	3.10
12-12	2.92	2.97	2.98	3.01	2.97
12-17	3.13	3.10	3.10	3.02	3.08

[a] Each value is the average for 25 fruits.

The breeding progenies were intermediate between the 2 extremes. Of them, 12-12 was consistently the shortest and 12-9 the longest.

Progeny 12-3 was discarded because fruits tended to taper too abruptly toward the blossom end. Progeny 12-17 was discarded because it was lowest in yield of the 4 breeding progenies, and no better in fruit type than 12-9 and 12-12. Evaluation of 12-9 and 12-12 was continued in 1954 at Madison and in commercial acreages at several locations in Wisconsin.

Determinations were made of percentages of mottled fruits, yields, and length/diameter ratios of standard varieties and of progenies 12-9 and 12-12 inoculated with cucumber virus 1 and grown in a plot at Madison in 1954 (Table 4). The length/diameter ratios of 1953 were very similar to those of 1954 and are included in Table 4. The average percentage of mottled fruits was very high in National Pickling and quite low in the resistant lines. It was slightly lower in 12-12 than in 12-9 or Ohio MR 17. There were no significant differences among yields of the resistant lines, while mosaic caused a great reduction in yield of National Pickling. In commercial plantings in central Wisconsin in 1953 and 1954, lines 12-9 and 12-12 yielded satisfactorily and had satisfactory processing quality.

Homozygous-scab-resistant varieties show no scab in the field under central and northern Wisconsin conditions where susceptible varieties show severe infection of young fruits as soon as favorable environmental conditions prevail. When young seedlings of susceptible and resistant varieties are inoculated in the greenhouse at high humidity and temperatures of 17°C. those of the former are killed rapidly while those of the latter show very limited infection and recover promptly. Since the greenhouse assay is much more reliable than field tests, all progenies were tested extensively in the greenhouse during the course of the investigation. Progenies 12-3, 12-9, 12-12, and 12-7 were thus shown to be highly resistant to scab. In 1953 and 1954, commercial plantings of progenies 12-9 and 12-12 were made in central Wisconsin where scab was prevalent on susceptible varieties. The 2 progenies remained completely free from scab in these tests.

DESCRIPTION OF THE NEW VARIETIES.—Lines 12-9 and 12-12 have been increased and released as Wis-

TABLE 4.—*Yield and length/diameter ratio of fruit and percentage of mottled fruits from inoculated plants of resistant and susceptible varieties, Madison, Wisconsin, 1954*[a]

Variety or progeny	Yield bu./acre	Length/diameter ratio		Average percentage of mottled fruit	Percentage of diseased fruits in grade indicated at last picking		
		1954	1953		Mild mottle	Severe mottle	White pickle
National Pickling	130	2.79	2.87	64	2	18	59
Ohio MR 17	304	3.14	3.15	16	16	0	0
12-9	294	3.10	3.10	13	6	0	0
12-12	278	2.91	2.97	5	11	0	0
LSD (19:1)	79			22			

[a] Plants were inoculated with cucumber virus 1. Values for length/diameter ratio are based on 25 fruits; values for percentages of diseased fruits are from 75 fruits.

consin SMR 9 and Wisconsin SMR 12, respectively. These varieties differ from each other chiefly in the relative length/diameter ratio of fruits. This is an important feature in processing and it is for this reason that both lines were released to the trade. For some purposes a relatively short fruit as in SMR 12 is desired, whereas for other purposes a somewhat slimmer fruit as in SMR 9 is needed. In vine type and season both varieties are very close to National Pickling. The fruits are black-spined. The fruit color of both varieties is lighter green than that of National Pickling, which in turn is lighter green than that of Ohio MR 17. Both varieties are quite close to National Pickling and Wisconsin SR 6 in season, in internal structure, and in solidity of fruit. Improvement of the double-resistant varieties in fruit color and internal structure can undoubtedly be made by outcrossing and reselection. Further breeding work with these objectives in mind is under way.

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TOXIN PRODUCTION BY HELMINTHOSPORIUM VICTORIAE [1]

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SUMMARY

Previous reports that in artificial culture *Helminthosporium victoriae* produces a toxic agent which induces the same disease symptoms and shows the same host specificity as does the pathogen itself were confirmed and extended. A chemically defined medium favorable for toxin production and a quantitative bioassay of the toxic agent based on retardation of root growth were developed. Culture filtrates, which at dilutions above 1/1,000,000 reduced root growth of oat seedlings of susceptible varieties 50 per cent, had no effect at a dilution of 1/10 upon seedlings of resistant oat varieties or of various garden vegetables and cereal grasses. Five mutant cultures, 3 that exhibited varying degrees of pathogenicity and 2 that failed to cause any symptoms characteristic of the disease were obtained from a single-spore isolate of *H. victoriae*. Among the pathogenic cultures, differences in pathogenicity correlated with differences in toxin production and these in turn were directly related to the growth rates of the 3 cultures. The growth rates of the 2 nonpathogenic cultures, neither of which produced appreciable quantities of toxin, were similar to those of the fastest and slowest growing pathogenic cultures. Tests of culture filtrates adjusted to various pH levels indicated that the toxic agent was quite stable at pH values below 4 but was unstable at higher pH levels and rapidly destroyed by heat if the reaction of the filtrate was neutral or alkaline. The high activity of the toxic agent, its specificity, and the association of its production with pathogenicity and growth in artificial culture all indicate that this toxin is primarily, if not solely, responsible for the symptoms which characterize this disease.

In its simplest form, the toxin theory states that disease symptoms in plants result from the direct action of specific toxic agents produced by the invading pathogen. Attempts to establish the validity of this theory have produced a mass of circumstantial evidence subject to various interpretations. Most of this evidence has been obtained from studies of toxic substances produced by plant pathogens in artificial

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culture. Doubt that such toxic substances play important roles in plant diseases is based on the fact that most of them exhibit one or more of the following characteristics: inability to induce all of the symptoms found in infected plants, failure to show host specificity comparable to that of the pathogen, production in larger quantities by weakly pathogenic or nonpathogenic strains than by highly pathogenic strains, or production in artificial culture only after periods much longer than those required for the development of disease symptoms in infected plants. Additional uncertainty regarding the role of toxins in plant disease has resulted from the lack of adequate methods for detecting toxins produced *in vivo* and distinguishing these from host reaction products. A discussion of these problems and of the need for more direct evidence regarding the toxin theory may be found in a series of review papers devoted to this subject (1, 2, 3, 5, 11).

It has been suggested that toxins produced by plant pathogens labeled with radioisotopes might allow the detection of toxins within host tissues and their separation from host reaction products, thus providing a direct test of the toxin theory (4, 11). Before experiments with labeled toxins can be carried out, considerable information on a suitable toxin-producing pathogen must be accumulated. A search of the literature indicated that *Helminthosporium victoriae* Meehan & Murphy, which causes severe damage to oat varieties possessing the Victoria type of resistance to crown rust, is well suited for such a study. In artificial culture, this pathogen produces a toxic agent which induces symptoms typical of the disease in oats and which exhibits a specificity similar to that of the pathogen (6, 7). It has also been shown that this pathogen can be labeled with radioactive carbon and still retain both its pathogenicity and its ability to produce the toxic agent (10, 11). The present report will deal with methods for producing high yields of the toxin, its specificity, a quantitative procedure for its bioassay, and the relation of growth and toxin production to pathogenicity of various isolates of this organism. A brief report of methods for isolation and partial purification of the toxin has been published (12).

MATERIALS AND METHODS.—Methods for production and bioassay of the toxin produced by *H. victoriae* were developed with an isolate supplied by J. G. Atkins, Jr. This isolate, which originally produced high yields of toxin and caused severe disease symptoms on susceptible varieties of oats, eventually lost both its toxin-producing ability and its pathogenicity. Further studies were carried out with cultures isolated during the spring of 1952 from naturally infected plants grown in the vicinity of Baton Rouge, Louisiana. Immediately after isolation, these cultures were tested for pathogenicity, and 10–15 single spores were isolated from each culture that caused typical disease symptoms. All experiments were conducted with cultures derived from these single-spore isolates. Stock cultures were stored at approximately 15°C in tubes of sterile soil, whereas cultures for routine laboratory use were maintained on oatmeal-agar slants at room temperature. Under the latter conditions, loss of pathogenicity and toxin-producing ability as well as changes in cultural characteristics often occurred. In nearly all such cases, the fungus could be recovered in its original form by reisolation from stocks stored in soil.

Bioassay of the toxin.—The results of a series of preliminary experiments confirmed previous reports (6, 7) that filtrates from cultures of *H. victoriae* contain a toxic substance that causes essentially the same disease symptoms as the pathogen itself. Susceptible varieties of oats treated with this toxic material show a striking reduction in growth of both roots and shoots (7, 12). Since tests revealed that roots were affected at much lower concentrations of the toxic agent than shoots, root elongation was used to develop a bioassay of the toxin. In this assay, oat grains of a susceptible variety were washed for 30 minutes in running tap water, placed in moist chambers, and incubated at 27°C for 42 hours after which grains having roots approximately 5 mm long were selected for uniformity. Five such grains were placed in a standard petri dish containing 5 ml of the material to be assayed. These were maintained for 72 hours at approximately 27°C and the longest root on each seedling was then measured.

In order to compare various lots of culture filtrates, the quantity of toxin in 1 ml required to reduce root elongation 50 per cent was designated as 1 milliunit, 1 unit being equal to 1000 milliunits. Levels of toxic activity were determined by assaying serial dilutions of toxic culture filtrates, plotting root lengths against the various dilution factors, and interpolating from the curve obtained the dilution factor required to retard root growth 50 per cent as compared to a distilled water check. This dilution factor divided by 1000 gave the number of units of toxin per ml.

Toxin production.—After a series of screening tests involving several types of natural and synthetic media and a variety of culture techniques, the following procedure was adopted as the most satisfactory one for obtaining high yields of the toxin produced by *H. victoriae*. Mycelial suspensions for seeding production flasks were prepared by transferring fungal material (together with a small amount of agar adhering to it) from oatmeal-agar slants to 250-ml Erlenmeyer flasks containing a number of pieces of broken glass rods and 25 ml of a modified Fries' medium[2]. These flasks were incubated at room temperature for 4 days after which they were placed on a mechanical shaker and thoroughly agitated for 30 minutes. During this process, the sharp edges of the broken glass cut

[2] This medium consisted of: 5 g $(NH_4)_2C_4H_4O_6$, 1 g NH_4NO_3, 1 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7\ H_2O$, 0.13 g $CaCl_2$, 0.1 g NaCl, 30 g sucrose, and distilled water to make 1 liter.

the mycelium into small bits, yielding a heavy mycelial suspension, 1 ml of which was used to seed each production flask. Production flasks of various sizes were used with highest yields of toxin being obtained from stationary cultures grown at 22–25°C on shallow layers of modified Fries' medium in small flasks. When grown on 20 ml of medium in 125-ml flasks, several of the isolates used in this study consistently yielded culture filtrates which assayed above 500 units per ml and in exceptional cases filtrates assaying above 5000 units per ml were obtained. In larger flasks, yields were considerably lower; for example, with 200 ml of medium in 2-liter flasks the most toxic culture filtrates obtained assayed slightly above 100 units per ml.

Unless otherwise specified, culture fluids to be assayed for toxic activity were first partially freed of fungal material by passage through several layers of cheesecloth. They were then autoclaved for 15 minutes at 17 lb. pressure and filtered through Whatman no. 1 filter paper. A few assays were conducted with culture filtrates diluted with modified Fries' medium lacking sucrose rather than with distilled water. Since similar results were obtained with both diluents all further tests were conducted with filtrates diluted with distilled water.

EXPERIMENTAL RESULTS.—*Toxin specificity.*—Previous investigators (6, 7, 8) have reported that oat varieties possessing the "Victoria type" of resistance to crown rust were much more severely damaged by *H. victoriae* and by the toxin produced by this pathogen than were oat varieties of other types. Tests involving 5 Victoria type varieties (Fulgrain, Victorgrain, Victorgrain 48-93, Victoria, and Arlington) and 4 non-Victoria type varieties (Nortex, New Nortex, Fergerson, and Camellia) confirmed these observations. Grains of each of these 9 varieties were used to assay a single lot of filtrate from a culture of *H. victoriae*. With the 5 Victoria varieties, all of which were equally sensitive, 50 per cent reduction in root growth was obtained at a 1/1,200,000 dilution of the culture filtrate. The other 4 varieties were all relatively nonsensitive, a 50 per cent reduction in root length occurred only at dilutions of 1/6 or less. A typical example of the striking difference in the sensitivity of the 2 types of varieties to the toxin produced by *H. victoriae* is shown in Figure 1.

Nothing was found in the literature regarding the effects of culture filtrates of *H. victoriae* on plants other than oats. Tests with a number of cereals (wheat, rye, barley, rice, and corn) and with garden vegetables (lettuce, tomato, pea, beet, carrot, cucumber, okra, radish, mustard, bean, and spinach) revealed that all of these plants reacted to the toxin in essentially the same manner as nonsensitive varieties of oats. Although no tests were made with microorganisms, bacteria and a number of species of fungi were observed growing vigorously in undiluted culture filtrates of *H. victoriae* during the course of various experiments.

Growth, toxin production, and pathogenicity.—A series of preliminary tests indicated that the pathogenicity of various isolates of *H. victoriae* was closely related to their ability to produce the toxic principle in artificial culture. To study this relationship, 5 isolates that varied in pathogenicity were selected (Fig. 2). These 5 isolates, designated A, B, C, D, and E, were all derived from a single-spore culture obtained from a naturally infected plant of Victorgrain 48-93. They were used in an experiment in which growth, pH changes in the culture medium, toxin production, and pathogenicity were determined. Mycelial suspensions of each of the 5 isolates were used to seed 500-ml production flasks containing 50 ml of modified Fries' medium. Growth, pH, and toxin determinations were begun 5 days after seeding and repeated at 2-day intervals thereafter. Growth determinations were made by removing, washing, drying, and weighing the mycelial mats from 4 flasks representing each isolate. At the same time the pH of a composite sample from the 4 flasks was determined with a Beckman potentiometer and a similar sample was assayed for toxic activity using oat grains of Victorgrain 48-93.

Inoculum for pathogenicity tests was prepared by separately fragmenting 20 g of mycelium from each isolate in 600 ml of water in a Waring Blendor. Fifty ml of inoculum was mixed with 50 oat grains which then were planted in a 4-in. pot of soil. A total of 6 replications were employed for each isolate using oat grains of susceptible (Victorgrain 48-93) and resistant (Camellia) varieties. The number of surviving plants in each pot was determined at 5-day intervals for a period of 30 days.

The results of this experiment are shown in Figures 3–6. The pathogenicity test (Fig. 6) confirmed previous results (Fig. 2) which indicated that isolate C was highly pathogenic, A somewhat less so, D slightly pathogenic, while B and E were nonpathogenic to plants of Victorgrain 48-93. None of the isolates caused a significant reduction in the number of plants of the Camellia variety. Furthermore, none of the inoculated plants of the resistant variety showed symptoms (basal necrosis or leaf striping) that characterize diseased plants.

The results presented in Figures 3, 5, and 6 show that isolates C, A, and D ranked in the same order in growth rate and toxin production as they did in pathogenicity. With these 3 isolates, maximum yields of toxin occurred shortly after the mycelial weight reached a maximum, and fluctuations in growth rates during the entire period were immediately reflected by fluctuations in yields of toxin. These results indicated that differences in pathogenicity among these 3 isolates were directly related to differences in the quantity of toxin produced and the time required for its production which in turn was a direct function of the growth characteristics of the isolates. On the other hand, lack of toxin production by the nonpatho-

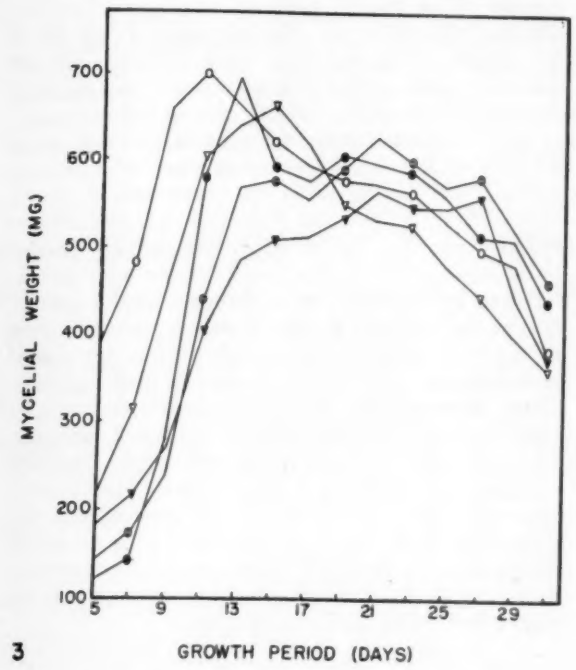
genic isolates (E and B) could not be attributed to inability to grow since the growth rate of E was intermediate to that of A and C, while B grew only slightly slower than D. The most toxic filtrates produced by isolates B and E assayed less than 0.01 units per ml—a value too low to be plotted on the scale used in Figure 5.

The pH values of the culture meduim (Fig. 4) varied inversely with the growth of the isolates and also with the quantity of toxin produced by the 3 pathogenic isolates. The toxicity of culture filtrates, however, could not be attributed to acid production *per se*, since isolate E which produced insignificant amounts of toxin yielded pH values essentially the same as those obtained with the high toxin-producer, isolate C. The most striking feature of the pH curves was the sharp rise that occurred near the end of the test period and coincided with an almost complete loss of toxic activity in the cultures filtrates of A, C, and D (Fig. 4, 5).

Stability of the toxic principle.—At the outset of these studies, several lots of culture filtrate with pH values ranging from 2.4 to 3.5 were autoclaved at 17 lb. pressure for periods up to 2 hours. These, assayed immediately after autoclaving, showed no loss in toxic activity; hence, all culture filtrates were

FIG. 1–2.—Fig. 1. Response of 2 varieties of oats to various dilutions of a culture filtrate of *H. victoriae*. Top row, Victorgrain 48-93 (susceptible); bottom row, Camellia (resistant).—Fig. 2. Effects of 4 isolates (A, C, D, and E), obtained as variants from a single-spore culture of *H. victoriae*, on seedlings of the oat variety Victorgrain 48-93. Isolate B (not shown) produced effects similar to those obtained with E. G is a noninoculated control.

routinely autoclaved at 17 lb. pressure for 15 minutes to destroy any fragments of mycelium which might be present. In view of the loss of toxic activity which occurred as the pH of the culture medium rose (Fig. 4, 5) it seemed desirable to study the stability of the toxic agent at various pH levels. Two series of tests were conducted. In the first of these, aliquots from a single lot of culture filtrate with a pH of 3 were adjusted to pH 4, 6, 8, and 10 with NaOH solution. These were stored at 10°C for 8 days during which



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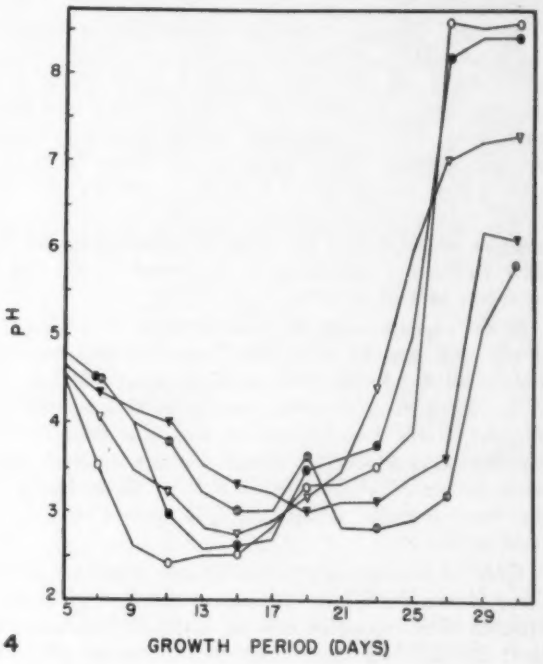

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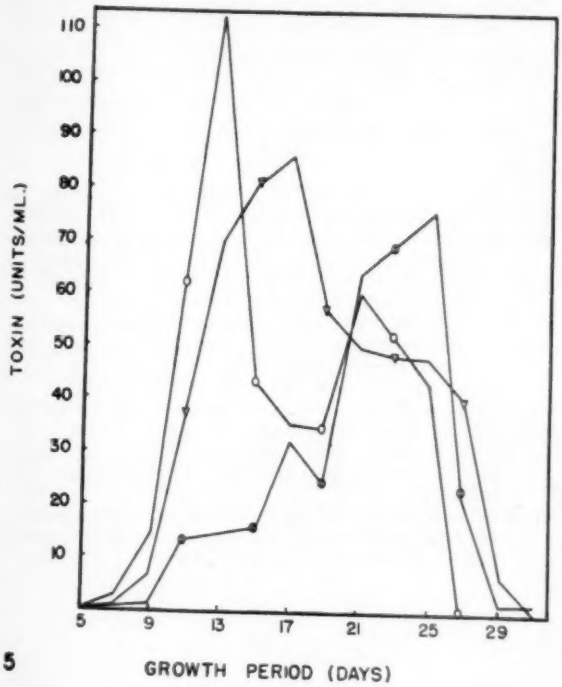

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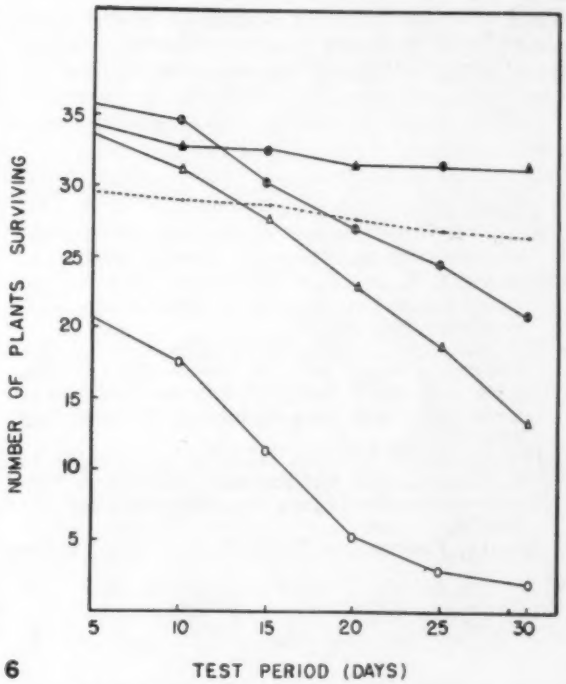

6

Fig. 3–6. Growth, pH changes, toxin production, and pathogenicity of isolates of *H. victoriae*. Open triangles, isolate A; solid triangles, B; open circles, C; barred circles, D; solid circles, E. Broken line in Figure 6 represents a noninoculated control.

they were assayed daily. During this period, samples with pH values of 3 and 4 showed no loss in activity. At pH 6 a very slight loss was noted after 5 days, at pH 8 about 50 per cent was lost after 1 day with less than 10 per cent remaining at the end of the 8-day period, and at pH 10 a 90 per cent loss occurred immediately with complete loss occurring after 4 days of storage. (Complete loss of activity was assumed if the filtrate failed to exhibit greater toxicity to Victorgrain 48-93 oats than to Camellia oats). This test was repeated with duplicate samples which had been autoclaved (17 lb. pressure for 15 minutes) after pH adjustment. With these, slight losses occurred after 5 days at pH 3 and 4, while at pH 6 and 8 the losses were similar to those observed in nonautoclaved samples at pH 8 and 10. Samples adjusted to pH 10, then autoclaved and assayed immediately, showed a complete loss of activity.

In the second series of tests, samples were adjusted to pH 6, 8, and 10 with NaOH solution and immediately readjusted with HCl solution to the original pH of 3. When such samples were assayed immediately, no appreciable loss of activity was observed. If the samples were heated or stored for a period of time, losses occurred that were similar to those found in the first test in which the pH values were not readjusted.

Effect of filtrates on resistant hosts.—Results, such as those shown in Figure 1, demonstrated that culture filtrates of *H. victoriae* can be obtained which are at least 100,000-fold more toxic to hosts susceptible to this pathogen than to those which are resistant. This indicates that such filtrates contain at least 1 toxic substance that is highly specific in nature. Several lines of evidence indicate that a second, nonspecific factor is primarily responsible for the low order of toxicity which these filtrates show to resistant hosts. First, filtrates from nonpathogenic isolates, such as B and E, cause 50 per cent retardation in root growth of both susceptible and resistant plants at dilutions of about 1/5 and are as toxic to resistant plants as are filtrates from highly pathogenic cultures. Second, filtrates adjusted to an alkaline reaction and heated for a prolonged period show a loss of more than 99.9 per cent in toxic activity when assayed with susceptible plants but remain as toxic to resistant plants as the original untreated material. Third, it was found possible to remove the nonspecific toxin without destroying the specific toxin. A crude culture filtrate, which caused 50 per cent reduction in root growth of resistant plants at 1/5 dilution, was partially purified by adsorption on charcoal and elution with acetone followed by separation on a chromatographic column. One of the fractions recovered was as toxic to susceptible plants as the original crude filtrate but showed no toxicity to resistant plants even in undiluted form. These observations strongly indicate that culture filtrates of *H. victoriae* contain at least 2 toxic substances: 1 that is highly active and specific for plants susceptible to the fungus and a second that is much less active and nonspecific. It is possible that this nonspecific toxic factor is responsible for the minor symptoms such as leaf flecking which several investigators (6, 8, 9) have reported to occur on resistant plants inoculated with *H. victoriae*.

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LITERATURE CITED

1. Allen, P. J. 1953. Toxins and tissue respiration. Phytopathology **43**: 221-229.
2. Bloch, R. 1953. Defense reactions of plants to the presence of toxins. Phytopathology **43**: 351-354.
3. Dimond, A. E., and P. E. Waggoner. 1953. On the nature and role of vivotoxins in plant disease. Phytopathology **43**: 229-235.
4. Hoffman, P. F., and B. M. Zuckerman. 1954. Oak wilt fungus labeled with C^{14}. Science **120**: 106-108.
5. Hollis, J. P. 1953. An approach to the physiology of toxin formation in microorganisms. Phytopathology **43**: 355-359.
6. Litzenberger, S. C. 1949. Nature of susceptibility to Helminthosporium victoriae and resistance to Puccinia coronata in Victoria oats. Phytopathology **39**: 300-318.
7. Meehan, Frances, and H. C. Murphy. 1947. Differential phytotoxicity of metabolic by-products of Helminthosporium victoriae. Science **106**: 270-271.
8. Paddock, W. C. 1953. Histological study of susceptpathogen relationships between Helminthosporium victoria M. and M. and seedling oat leaves. N. Y. (Cornell) Agr. Exp. Sta. Memoir 315, 63 p.
9. Rosen, H. R. 1954. Variation in pathogenicity in the Helminthosporium blight organism of oats and evidence for its relationship to H. sativum. U. S. Dept. Agr. Pl. Dis. Rptr. Sup. **228**: 114-115.
10. Wheeler, H. E. 1952. The use of radiocarbon for tagging fungi. Phytopathology **42**: 431-435.
11. Wheeler, H. E. 1953. Detection of microbial toxins by the use of radioisotopes. Phytopathology **43**: 236-238.
12. Wheeler, H. E., and H. H. Luke. 1954. Studies of a toxic agent produced by Helminthosporium victoriae. (Abs.) Phytopathology **44**: 334.

THE RELATIONSHIP OF ROOT-KNOT NEMATODES TO BLACK-SHANK RESISTANCE IN TOBACCO [1]

J. N. Sasser, G. B. Lucas, and H. R. Powers, Jr.

SUMMARY

In greenhouse experiments, plants of 2 varieties of black-shank-resistant tobacco were grown in steamed soil to which *Phytophthora parasitica* var. *nicotianae* and *Meloidogyne* spp. were added alone and in combination. Those plants inoculated with the black-shank fungus in the presence of root-knot nematodes developed black-shank symptoms earlier and to a greater extent than did plants grown in soil infested with only the black-shank fungus. The role of the nematodes appears to be more than the wounding of root tissues alone, since plants the roots of which were cut and inoculated immediately or at later intervals with the black-shank fungus had no greater incidence of black shank than uninjured inoculated plants. These experiments indicate that until nematode-resistant tobacco varieties are available it will be advisable to control root knot either by soil fumigation or crop rotation in fields where the black-shank fungus also is present.

Two of the principal diseases of tobacco (*Nicotiana tabacum* L.) in North Carolina are Granville wilt (caused by *Pseudomonas solanacearum* E. F. Sm.) and black shank (caused by *Phytophthora parasitica* Dast. var. *nicotianae* (B. de Haan) Tucker). In 1949, varieties of flue-cured tobacco resistant to both black shank and Granville wilt were released in North Carolina. By 1951, 1 of these varieties (Dixie Bright 101, which has moderate black-shank resistance) was being grown successfully on a large acreage. In some fields, however, this variety did not grow well, and a high percentage of the plants were killed by black shank. Often such fields were found to be heavily infested with 1 to several species of parasitic nematodes: root-knot nematodes (*Meloidogyne* spp.), meadow nematodes (*Pratylenchus* spp.), and the tessellate stylet nematode (*Tylenchorhynchus claytoni* Steiner).

Nusbaum and Chaplin[2] found that damage caused by black shank was greatly reduced when measures were taken to control nematodes in these fields by soil fumigation. They also showed, in greenhouse experiments, that the fumigants used were not fungicidal even at rates 10 times as high as those used in the field for nematode control. These observations provided strong circumstantial evidence that parasitic nematodes were contributing to the increased damage caused by black shank in certain fields.

No reports were found in the literature of experiments conducted under controlled conditions to investigate the possibility of interaction between root-knot nematodes and the black-shank fungus. Greenhouse experiments were initiated, therefore, to determine the specific role of the cotton root-knot nematode, *Meloidogyne incognita acrita* Chitwood, and other *Meloidogyne* spp. in altering the resistance of tobacco to black shank. A preliminary report has been published.[3]

MATERIALS AND METHODS.—Tobacco seedlings of variety Dixie Bright 101 (moderately black-shank resistant) or Dixie Bright 102 (highly black-shank resistant) were grown in thumb pots until they reached transplant size and were then repotted into 4- or 6-in. pots. The plants were divided into 4 groups and approximately 5 g of tomato or tobacco roots containing egg masses of the cotton root-knot nematode *M. incognita acrita*, was added to the steamed soil in each pot of 1 group of plants. In some of the preliminary tests, *M. incognita* and *M. javanica* were also used. Fifty ml of a water suspension of mycelial fragments from a 2-week-old oatmeal-agar culture of the black-shank fungus was poured over the root system in each pot of the second group of plants. A third group of plants had both pathogens added to the soil as indicated above, and a fourth group, the controls, was grown in steamed or methyl bromide fumigated soil in the absence of both pathogens. The experiment was repeated 6 times. Usually 20 plants were included in each treatment and the tests were conducted at greenhouse temperatures ranging from 21–27°C at night to 27–38°C in the daytime. Height measurements and numbers of plants with visible symptoms of black shank were made at weekly intervals.

To determine whether plants already infected with root-knot nematodes would be more severely damaged by the black-shank fungus than would healthy plants, Dixie Bright 101 seedlings were transplanted to steamed soil and treated in the following manner: 1) root-knot inoculum was added to the soil and the plants were allowed to grow for 30 days, or 2) plants to which no root-knot inoculum had been added were

[1] Accepted for publication April 1, 1955.
Contribution from Plant Pathology, North Carolina Agricultural Experiment Station, Raleigh, North Carolina. Published with the approval of the Director of Research as Paper No. 623 of the Journal Series.

[2] Nusbaum, C. J., and J. F. Chaplin. 1952. Reduction of the incidence of black shank in resistant tobacco varieties by soil fumigation. (Abs.) Phytopathology 42: 15.

[3] Sasser, J. N., H. R. Powers, Jr., and G. B. Lucas. 1953. The effect of root-knot nematodes (Meloidogyne spp.) on the expression of black shank resistance in tobacco. (Abs.) Phytopathology 43: 483.

allowed to grow for 30 days. The black-shank fungus was added to the soil of half of the plants in each treatment.

To determine the effect of mechanical root injury on black shank incidence, separate tests were conducted with a group of transplants in which the roots on 1 side of each transplant were cut with a sharp knife. Since decomposition products from rotting plant material may also injure living roots, approximately 5 g of chopped healthy tomato roots was placed under each plant of a second group of transplants. Fifty ml of a suspension of mycelial fragments of the black-shank fungus was then added to the soil of both groups at 1 of the following intervals: 1) transplanting time, 2) 1 day after transplanting, 3) 2 days after transplanting, and 4) 4 days after transplanting. The plants receiving these treatments were then compared with transplants with uncut roots which were inoculated with the black-shank fungus either at 1) transplanting time or 2) 1 day after transplanting.

Experimental results.—Within 1 week after transplanting, plants growing in soil to which both the black-shank fungus and the root-knot nematodes had been added were more stunted than those in the other treatments, and some of the plants were also wilted. This was true in all tests. In about 2 weeks a high percentage of the plants had black stem-lesions typical of black shank and were either dead or dying. In sharp contrast, at the end of 2 weeks a low percentage of the plants growing in soil infested only with *P. parasitica* var. *nicotianae* were showing black-shank symptoms (Fig. 1).

In a typical experiment, 75–100 per cent of the plants grown in soil infested with both pathogens were dead within 3 weeks after transplanting. In soil infested with only the black-shank fungus, however, the number of plants exhibiting black-shank symptoms ranged from 0–30 per cent (Fig. 2). Neither the control plants nor those growing in soil infested with root-knot nematodes had black shank.

Further evidence that the combination of the 2 organisms has a greater pathogenic effect than either alone is shown by the data based on height measurements. As shown in Figure 3, plants growing in soil infested with root-knot nematodes and the black-shank fungus were severely stunted, whereas those growing in soil containing only the latter pathogen grew almost as rapidly as the controls. Plants growing in soil infested with root-knot nematodes were taller than those growing in soil infested with both pathogens.

When Dixie Bright 101 tobacco plants were allowed to grow for 30 days in soil infested with root-knot nematodes prior to the addition of the black-shank fungus some plants developed black-shank symptoms within a week after the addition of the fungus. Root-

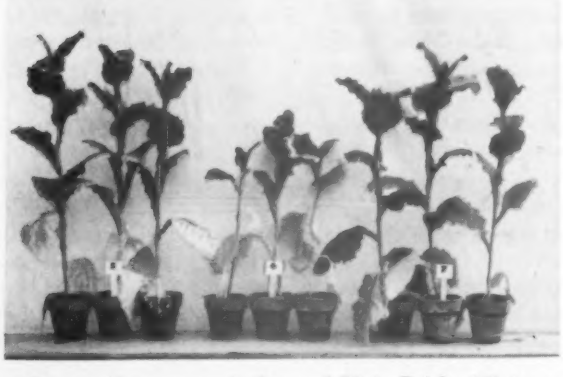

Fig. 1. Appearance of plants of Dixie Bright 101 inoculated at transplanting time. Left: inoculated with black-shank fungus only. Center: inoculated with both the black-shank fungus and root-knot nematodes. Right: inoculated with root-knot nematodes only. Note stem lesion on the plant to left of middle group.

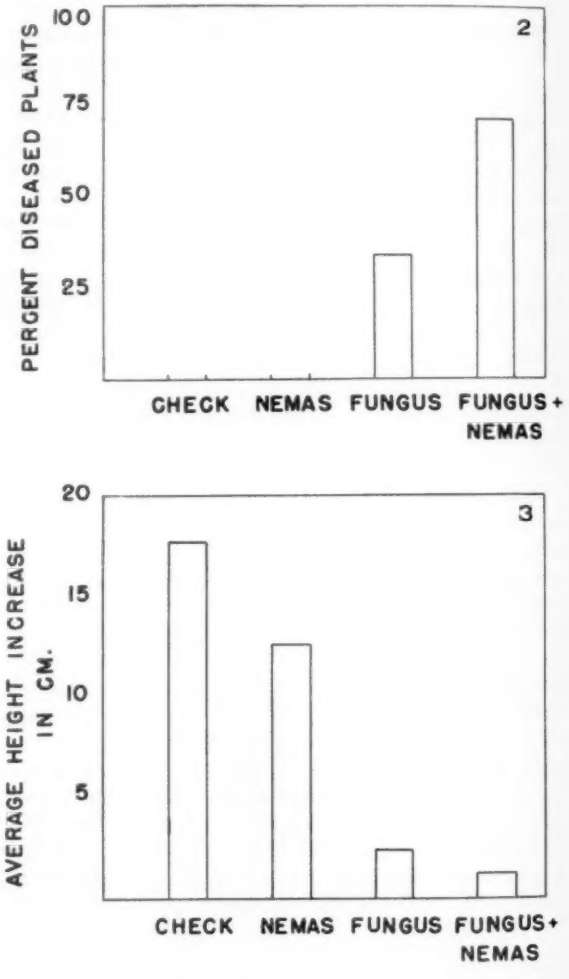

Fig. 2–3.—Fig. 2. Percentage of plants showing black shank symptoms after 3 weeks' growth in soil to which root-knot nematodes and the black-shank fungus had been added alone and in combination.—Fig. 3. Average increase in plant height of tobacco plants after 14 days' growth in soil to which root-knot nematodes and the black-shank fungus had been added alone and in combination.

knot-free plants of the same age also inoculated with the black-shank fungus showed no symptoms of black shank. One month after inoculation with the black-shank fungus, 80 per cent of the nematode-infected plants obviously were infected with the black shank fungus. Root-knot-free plants of the same age which had been inoculated with the black-shank fungus showed no black-shank symptoms, although they were noticeably smaller than the control plants and than plants growing in soil to which only the black-shank fungus had been added.

Cutting the roots or permitting chopped healthy tomato roots to decay in contact with the tobacco roots and then adding the black-shank fungus immediately or at later intervals did not increase the incidence of black shank.

DISCUSSION AND CONCLUSIONS. — In nematode-free soil, Dixie Bright 101 is moderately resistant and Dixie Bright 102 is highly resistant to black shank. These varieties, however, are susceptible to all the root-knot-nematodes species known in this country as well as to the meadow nematodes and the tessellate stylet nematode. Although nematodes rarely kill plants outright, they can cause considerable injury by feeding on the roots. It cannot be stated conclusively, however, that these injuries are the only factor involved in predisposing the plants to more severe damage by the black-shank organism, as it is known that wounds are not necessary for the black-shank fungus to enter the roots.[4] In addition, allowing the plants to become severely infected with the root-knot-nematode prior to the addition of the black-shank fungus increased the black-shank damage to the same degree as the addition of both organisms at time of transplanting.

In the case of the root-knot nematode on tobacco, the host-parasite reaction is complex. Necrosis of the tissue usually occurs only under conditions of mass invasion of the roots by the nematode. There is, however pronounced hypertrophy and hyperplasia that result in malformation of the roots. Such abnormalities obviously affect root functions, but the physiochemical effects of the secretions from the salivary glands injected into the host tissue by a large number of nematodes might even be more detrimental to the plant.

These experiments, conducted under partially controlled conditions, have shown that root-knot nematodes in combination with the black-shank fungus invariably increase the incidence of black shank. Furthermore, factors other than mechanical injury appear to be involved in the break-down of resistance. It may be that the nematodes contribute to some biochemical alteration of the host cells that makes them a more congenial substrate for the black-shank fungus. Whether or not such a complex relation exists awaits further study. It is evident, however, that until nematode-resistant tobacco varieties are available, it will be advisable to control root knot, either by soil fumigation or crop rotation, in fields where black shank is present. Otherwise, full value will not be received from the use of present day black-shank-resistant varieties.

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[4] Nusbaum, C. J. 1952. Host-parasitic relations of Phytophthora parasitica var. nicotinae in roots of resistant and susceptible tobacco varieties. (Abs.) Phytopathology 42: 286.

PHYTOPATHOLOGICAL NOTES

V-8 Juice Agar as a General-purpose Medium for Fungi and Bacteria. PATRICK M. MILLER.[1] A culture medium that will support growth or sporulation of widely diverse types of fungi and bacteria is a useful or necessary tool for workers in plant pathology. Potato-dextrose agar (PDA) is often used for this purpose; however, some fungi, such as *Stemphylium solani* Weber, sporulate poorly if at all on it.

Diener[2] found that a 20 per cent V-8 juice agar plus ultraviolet irradiation gave the best sporulation by *S. solani.* V-8 is the proprietary name of a product of the Campbell Soup Company and is a mixture of juices of tomato, carrot, celery, parsley, beet, lettuce, spinach, and watercress. In the past 3 years, however, good sporulation by *S. solani* on V-8 agar was obtained without the use of irradiation. The agar is prepared by combining 200 ml of V-8 juice and 3 g of calcium carbonate with the amount of agar desired and diluting with water to 1 liter. The pH of agar prepared in this manner is about 7.25, but the pH can be changed by varying the amount of calcium carbonate. In tests utilizing agar slants, *S. solani, Alternaria solani* (Ell. & G. Martin) Sor., *S. sarcinaeforme* (Cav.) Wiltsh., *S. botryosum* Wallr., *Septoria lycopersici* Speg., *Helminthosporium turcicum* Pass., and *Phytophthora in-*

[1] Formerly Assistant in Plant Pathology, Department of Horticulture, University of Illinois, and now Assistant Plant Pathologist, Connecticut Agricultural Experiment Station, New Haven, Connecticut.

[2] Diener, U. L. 1952. A method for inducing abundant sporulation of Stemphylium solani in pure culture. (Abs.) Phytopathology 42: 7.

festans (Mont.) D By. sporulated better on V-8 agar than on PDA. *Aspergillus niger* Michele, *Penicillium oxalicum* Thom, and *Monilinia fructicola* (Wint.) Honey sporulated equally well on V-8 agar and PDA. Sporulation by *S. sarcinaeforme* on V-8 agar was equal to that obtained on oat-meal agar. Sporulation by *P. infestans* on V-8 agar was almost equal to that on lima-bean agar. *Glomerella cingulata* (Ston.) Spauld. & Schrenk grew slightly better on PDA than on V-8 agar but it has been the only organism tested to do so.

In routine culture work at the University of Illinois and at the Connecticut Agricultural Experiment Station, V-8 agar was used successfully for the culture of a very diverse group of organisms. These include, besides those previously named, *Erwinia amylovora* (Burr.) Winsl. et al, *Xanthomonas campestris* (Pam.) Dows., *Corynebacterium michiganense* (E. F. Sm.) H. L. Jens., *Botryosphaeria ribis* Gross. & Dug., *Pleospora herbarum* (Fr.) Rab., *Physalospora obtusa* (Schw.) Cke., *Colletotrichum phomoides* (Sacc.) Chester (*Glomerella phomoides* (Sacc.) Swank), *Alternaria tenuis* Auct., and a very wide variety of soil fungi, including sclerotial and non-sclerotial types, and perfect and imperfect forms. The juice is usually used unfiltered, but for soil fungi it can be used successfully either filtered or unfiltered. Besides its versatility as a general media, V-8 agar is an inexpensive media—juice enough for 1 liter of agar costing less than 7 cents.—University of Illinois and the Connecticut Agricultural Experiment Station.

An Extensive Type of Necrotic Host Reaction of Oats to Crown Rust.[1] M. D. SIMONS. Necrotic areas on the leaves of the host plant are common symptoms of infection of oat seedlings by the crown rust organism (*Puccinia coronata* Cda. var. *avena* Fraser & Led.) in the greenhouse. This type of reaction is regarded as a manifestation of resistance to the pathogen. The appearance of the lesions varies, depending upon the interaction of host variety, race of the parasite, and environment. The affected host tissue may range in reaction from simple discoloration to extensive shrinkage and distortion.

Murphy[2] described 7 types of host reaction to crown rust, 3 of which were characterized by the appearance of "necrotic areas." The extent of these areas was not mentioned, but it was stated that the infection types were similar to those described for stem rust by Stakman, Levine, and Bailey.[3] The illustrations presented by these investigators showed no extensive necrosis, and the small necrotic areas were described as being

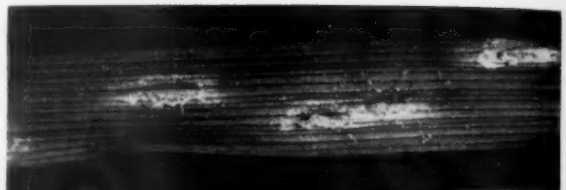

FIG. 1. Upper: typical reaction of Bond oats to crown rust race 202. Lower: characteristic necrotic reaction of Bond to certain cultures of crown rust race 258.

"sharply defined." Recent illustrations by Finkner[4] of the crown rust infection types, including those typical of the Victoria variety, also suggest that a necrotic lesion resulting from penetration of a single urediospore is limited to a small area around the infection court.

In the course of identifying collections of crown rust made in 1951, it was observed that infected leaves of the differential variety Bond were sometimes either completely dead or that they contained large, irregular areas of dead tissue (Fig. 1). Uredia often were present in the living sections and occasionally in some of the necrotic areas of infected leaves. The size and vigor of these uredia were more or less inversely proportional to the size and to the degree of distortion of the necrotic area. Previously, leaves of the old crown rust differential variety Sunrise often had shown somewhat similar symptoms. It was assumed, however, that the leaves of Sunrise were merely unusually susceptible to injury from high temperature or other unfavorable conditions in the humidity chamber. As Bond exhibited this reaction under a wide range of conditions, including conditions regarded as optimum, it was evident that the large necrotic areas were the result of something other than unfavorable environment. The general appearance of the necrotic lesions suggested that they might be due to the action of some facultative parasite such as *Septoria avenae* Frank. In subsequent inoculations, however, cultures increased from single well-isolated uredia occurring on leaves showing no necrosis would induce as much necrosis as the parent culture. The cultures inducing this reaction in Bond were relatively common, and the necrotic lesions have been observed only on Bond or Bond-derived varieties. None of the other symptoms associated with other common diseases of oats, such as the

[1] Journal Paper No J-2664 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 1176.

[2] Murphy, H. C. 1935. Physiologic specialization in Puccinia coronata avenae. U. S. Dept. Agr. Tech. Bul. 433, 48 p.

[3] Stakman, E. C., M. N. Levine, and D. L. Bailey. 1923. Biologic forms of Puccinia graminis on varieties of Avena spp. Jour. Agr. Res. 24: 1013-1018.

[4] Finkner, V. C. 1954. Genetic factors governing resistance and susceptibility of oats to Puccinia coronata var. avenae, F. and L., race 57. Iowa Agr. Exp. Sta. Res. Bul. 411: 1040-1063.

pycnidia of *S. avenae* or the halos or exudates associated with common bacterial pathogens were ever observed in or near these lesions. Consequently, it was concluded that the large lesions were the result of infection by the crown rust organism.

Attempts to isolate a pure culture of the rust organism that would consistently induce necrosis without the formation of susceptible-type uredia anywhere on the leaves of Bond have so far been unsuccessful. This variable reaction type on a single leaf of Bond, ranging from fully susceptible 4-type uredia to large, necrotic, shriveled areas containing no uredia, appears to be an inherent characteristic of this particular host-parasite interaction. It should be noted, however, that the sporulating uredia were almost always few in number, and that it was very difficult or impossible to maintain these cultures from generation to generation on the Bond variety.

Data obtained from the identification of crown rust collections made in 1952 and 1953 indicated that cultures that induced this extensive necrotic reaction in Bond usually induced susceptible or moderately susceptible reactions in the Victoria variety. When relative spore production was used as the criterion of reaction type, Bond was classified as "resistant" to these cultures, most of which then keyed out to race 258.[5]—Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture and Iowa Agricultural Experiment Station.

[5] Simons, M. D., and H. C. Murphy. A comparison of the relative efficiency of certain combinations of oat varieties in differentiating races of crown rust. U. S. Dept. Agr. Tech. Bul. (In press).

LISTING OF BOOKS

PHYTOPATHOLOGY will, from time to time as space becomes available, list titles of books on pathological subjects. A brief description of the contents of the books will be included.

BOYCE, J. S. 1954. **Forest plantation protection against diseases and insect pests.** F.A.O. Forestry Development Paper No. 3. Food and Agriculture Organization of the United Nations. Rome, Italy. 41 p. Price $0.50–2s. 6d.

A critical summary of the world literature relating to disease and insect problems in forest plantations. The author emphasizes the lessons to be learned from the older plantations in western Europe, where forest planting first began. There is a bibliography of 47 English and 24 foreign titles. The author is Professor of Forest Pathology at Yale University.

WESCOTT, CYNTHIA, PETER K. NELSON, AND EDITORIAL COMMITTEE OF THE BROOKLYN BOTANIC GARDEN, ED. 1955. **Handbook on pests and diseases.** Brooklyn Botanic Garden, Brooklyn 25, N. Y. 96 p. Price $1.00.

This special printing of PLANTS AND GARDENS consists of 12 illustrated articles by eminent plant pathologists and entomologists. The material presented is limited to the common diseases and insect pests of basic ornamental plantings: shrubs, small trees, roses, lawns, a few perennials, and a few house plants. An index lists the pests and diseases described and tells how each can be recognized.

ABSTRACT OF A PAPER PRESENTED AT THE 1955 ANNUAL MEETING OF THE SOUTHERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

This abstract was omitted inadvertently from the list of abstracts published together with the report of the meeting (PHYTOPATHOLOGY 45: 346-350).

Development of burley varieties of tobacco resistant to black shank, Fusarium wilt, wildfire, tobacco mosaic, and black root-rot. HEGGESTAD, H. E., AND E. E. CLAYTON. Tobacco varieties Burley 11A and Burley 11B, recently developed and released, have combined resistance to black shank, caused by *Phytophthora parasitica* var. *nicotianae;* and black root-rot, caused by *Thielaviopsis basicola.* Burley 11B is slightly more resistant to all of the diseases than Burley 11A. Average results from 18 replicated variety tests conducted in 1953 in absence of disease showed Burley 11A to be superior to commonly grown varieties in quality and about equal in yielding ability. Burley 11B was somewhat inferior to Burley 11A in yield and quality. A new variety, Burley 21, also has been developed with combined resistance to wildfire (caused by *Pseudomonas tabaci*), tobacco mosaic, and black root-rot. The wildfire resistance was derived from *Nicotiana longiflora* and mosaic resistance from *N. glutinosa.* Burley 21, tested extensively as Gr. 25, possesses unusual seedling vigor and excellent "stand-up" growth habit. Yields and quality characteristics are good compared with commonly grown varieties. Several other wildfire-mosaic resistant breeding lines, Gr. 26, Gr. 27, Gr. 28, and Gr. 29, were inferior to Burley 21 in quality of cured leaf.

REPORT AND ABSTRACTS OF THE 1954-55 ANNUAL MEETING OF THE POTOMAC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The twelfth regular meeting of the Potomac Division of the American Phytopathological Society was held March 3 and 4, 1955, in the auditorium of the Plant Industry Station, Beltsville, Maryland. Approximately 200 persons attended.

Twenty-one scientific papers were presented. The annual banquet was held in the Plant Industry Station cafeteria. Following the banquet, Dr. Karl D. Butler, Farm Counselor, Avco Corporation, spoke on "The future of agriculture." During the second day a symposium on "Fungicides—past, present, and future" was held with Dr. J. W. Heuberger as chairman. The topics and speakers were: 1) "History and philosophy of the use of fungicides"—G. L. McNew; 2) "The mode of action of fungicides"—Hubert Martin; 3) "Problems encountered in the commercial production and formulation of fungicides"—T. Walter Reed; and 4) "Legal and moral aspects encountered in the use of fungicides—the Miller Bill"—Richard J. Both.

The following officers were elected to serve during the next year: *President,* H. Rex Thomas; *Vice-President,* J. G. Leach; and *Councilor,* W. D. McClellan.

L. O. Weaver, *Secretary-Treasurer.*

ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

Control of an ectoparasitic nematode injurious to deciduous fruit trees. Adams, Robert E. Young peach trees growing in soil treated with 30, 36, and 60 oz. of benzene hexachloride (BHC) on 100 sq. ft. of soil showed increased growth over untreated trees apparently due to the demonstrated control of *Xiphinema* sp., an ectoparasitic nematode. A treatment of 90 oz. BHC proved toxic to the trees. Excepting the 90-oz. treatment, there was a significant negative correlation between tree growth and nematode population. Injury to established apple trees by this nematode is a contributing factor to the condition known locally as "pout." One season's treatment with BHC improved the appearance of such apple trees, reduced the nematode population in the soil, and caused no abnormal flavor in the fruit.

Two sap-transmissible viruses from carnation. Brierley, Philip, and Floyd F. Smith. Two distinct viruses of carnation have been confused in research publications. Carnation mosaic virus, which is transmissible by sap and by *Myzus persicae,* produces mottling in 10 species of the pink family, but fails to infect *Gomphrena globosa,* tobacco, cucumber, bean, or China aster. Carnation ringspot virus is sap-transmissible to Gomphrena and from Gomphrena to tobacco, cucumber, bean, China aster, and other plants. Ringspot virus has no known insect vector and has a higher tolerance to heat and dilution than mosaic virus. Mosaic virus is separable from mixed infections by means of transmission by *Myzus persicae* to healthy *Dianthus barbatus* or other susceptible pinks, and ringspot virus is separable by passage through Gomphrena. Our mosaic virus is the carnation mosaic virus of Ames and Thornberry; our ringspot virus appears to be the mosaic virus of Noordam, Thung, and van der Want.

Leaf variegation in strawberry. Darrow, Geo. M. Leaf variegation has been found in the diploid species *Fragaria vesca,* in the hexaploid *F. moschata,* in 3 octoploids, *F. virginiana, F. chiloensis,* and *F. ovalis,* and in many horticultural varieties. A causal agent has not been transmitted by grafting or by insects. The disease appears in the cotyledon stage of selfed seedlings as albinism and in hybrid progenies as chlorosis of true leaves at any later time. In 1 season, variegation appeared in Blakemore throughout the United States. In the Blakemore and Howard 17 varieties, nonvariegating stocks have largely replaced the variegating ones, and variegation is not now a serious trouble in the United States, except in the Wisconsin 537 variety, of which a nonvariegating stock has been named Sharon. In Great Britain, the leading variety Climax is variegating and many fear that it may be lost. Variegation is considered to be due to an unstable ever-mutating gene widely present in the genus *Fragaria.* Variegated seedlings have been obtained by irradiating seed of *F. vesca.* Use of nonmutating or relatively nonmutating varieties in breeding seems effective for control of variegation.

Physiologic races of Phytophthora infestans; their use in breeding for resistance. Gallegly, M. E. Of 16 potato races of *P. infestans* identifiable on differential hosts with genes R_1, R_2, R_3, and R_4, only races 1,3 and 2,3 are missing from the West Virginia collection. Genotypes of selections from crosses involving the 4 genes are determined by separate inoculations with races 1,2,3; 1,2,4; 1,3,4; and 2,3,4. A mixture of these races will kill all individuals except those with the 4 genes. Inoculations of *Solanum* spp. and F_1 hybrids from *S. demissum* $\times$ 2n *Solanum* spp. with the above races and with race 1,2,3,4 show that *S. demissum* has at least 2 additional dominant resistance genes. By use of these genes, the isolate thought to be race 1,3,4 proved to be a new race (1,3,4 +, e.g. 1,3,4,5). Tomato isolates of the fungus were found to be either tomato race 0 or tomato race 1. Isolates within a tomato race varied in virulence. Plants with the dominant TR_1 gene were resistant to all isolates of race 0 but were susceptible to those of race 1. Plants with multiple genes only showed resistance to isolates of either race low in virulence, but they may be susceptible to more virulent ones. Judicious use of these isolates has resulted in tomato selections of commercial types bearing both TR_1 and multiple-gene resistance.

Pathological effects of a spiral nematode on boxwood roots. Golden, A. Morgan. In a study of boxwood roots experimentally inoculated with a new species of *Helicotylenchus,* the over-all effect of this ectoparasitic nematode on the root system was that of stunting and production of brownish discoloration. Microscopic examinations revealed numerous tiny brown necrotic lesions on the smaller roots. These were concentrated in some areas more than in others. A lesion sometimes involved only 1 or 2 cells on the surface, but more often several cells leading well into the cortex of the root were involved. Generally, a few cells around and beyond the point of actual penetration by the nematode showed a reaction to safranin-fast-green stain different from that of unaffected cells. It appears that this nematode causes direct injury to the roots in 3 ways: 1) it mechani-

cally destroyed the cells and left punctures in the roots; 2) it changed the cell contents chemically, probably by secreting digestive substances while feeding; and 3) it removed cell contents as food.

Wild sources of blueberry stunt virus in New Jersey. HUTCHINSON, M. T., A. C. GOHEEN, AND E. H. VARNEY. Blueberry stunt, a virus disease, was described in 1942 on diseased plants in cultivated blueberry fields. In 1952 plants of *Vaccinium vacillans*, the wild dryland lowbush blueberry, developed symptoms of virus infection when grafted with scions of cultivated blueberry infected with stunt virus. A survey indicated that similar virus symptoms were widely spread through the native population of *V. vacillans* in all areas of New Jersey. It also showed that stunt-like symptoms were present in wild *V. corymbosum*, *V. atrococcum*, and *V. stamineum* throughout the range of these species in New Jersey. Virus was transmitted from cultivated and wild *V. corymbosum* and *V. vacillans* to *Vinca rosea* by means of dodder (*Cuscuta subinclusa*). In Vinca, virus from all 3 sources produced identical symptoms. Virus was further transmitted from these to other Vinca plants by budding. Subsequently, stunt virus was transmitted by grafts and buds from diseased wild *Vaccinium corymbosum*, *V. atrococcum*, *V. stamineum*, and *V. vacillans* to healthy *V. corybosum* (variety Jersey) indicator plants. The widespread distribution of stunt virus in wild species of *Vaccinium* in New Jersey indicates that the virus is indigenous there and that it was spread by its vector into the first cultivated blueberry fields.

Studies on the use of antibiotics for the control of bacterial spot of pepper. KRUPKA, L. R., AND D. F. CROSSAN. Field observations in 1954 indicated that infection could reoccur on pepper plants that had been sprayed with varying concentrations and applications of Agri-mycin (15 per cent streptomycin; 1.5 per cent oxytetracycline) and crude streptomycin. Spray studies were initiated in the greenhouse and laboratory to determine if Agri-mycin was 1) translocated into pepper leaves, 2) merely a surface protectant, or 3) acting as an eradicant. In tests in which a plate bioassay technique was used to determine the presence or absence of the antibiotic after spraying, it was found that expressed juice from washed pepper leaves did not contain detectable amounts of the antibiotic. Bioassays made daily for 7 days from plants that were provided with periods of free moisture to simulate dew on the leaf surface, showed that Agri-mycin (250 ppm) sprayed on leaf surfaces was detectable in unwashed leaf samples at 5 days but not at 10 days after application. In artificial inoculations, Agri-mycin-sprayed (250 ppm) plants afforded a moderate degree of protection from infection when the inoculum was applied within 5 days after application of the antibiotic. Application of 3 consecutive sprays of Agri-mycin (500 ppm) failed to eradicate the organism from infected pepper leaves.

Nematode attacking soybean in Brazil. LORDELLO, LUIZ GONZAGA E. One of the most serious detriments to soybean cultivation in the State of São Paulo, Brazil, is infection by meadow and root-knot nematodes. Of these 2, the root-knot nematodes are best known to the growers. Investigations have been carried out to select resistant varieties that could be cultivated on a large scale or used in breeding work. The variety La. 41-1219, introduced from the United States as one that is susceptible in Mississippi, was injured by *Meloidogyne incognita* but resistant to a closely related form to be elsewhere described as a new species. The variety N 46-2652, introduced as a resistant strain, was injured by the new species of nematode. The variety Abura, one of the most widely cultivated varieties, was attacked by the new species and also by an undescribed subspecies of *M. javanica*. The 3 members of the genus *Meloidogyne* referred to in this paper are the root-knot forms involved in the soybean problem up to the present time.

Sclerotia production in Stromatinia gladioli as affected by potassium and other inorganic nutrients. MARSHALL, B. H., JR. To determine the effects of several elements on its growth, *Stromatinia gladioli* was grown on a mineral-sucrose solution for periods of 14 and 28 days. At the end of these periods, the fungus mats were harvested, dried, weighed, and examined for sclerotia. Two levels each of N (14 and 140 ppm) and of P (9.3 and 93 ppm) were used in the nutrient solutions. K, Mg, and B were present at several different concentrations. Of the elements tested, K had the most influence on the formation of sclerotia. It speeds sclerotia production and at a high level (78 ppm) provides for the production of approximately 50 per cent more sclerotia than at lower levels (7.8 and 39 ppm). An increase in K above 78 ppm had no effect on sclerotia. Mg and P also had decided effects on sclerotia, but the effect of B was very slight. N seemed to affect the size of sclerotia, but not their formation. Where N was low, sclerotia were numerous but rudimentary.

Some responses of fungi to light. MCCLELLAN, W. D., H. A. BORTHWICK, IDA BJORNSSON, AND BARTON H. MARSHALL, JR. Thirteen different fungi have been screened for gross responses to filtered and unfiltered warm white fluorescent and mazda light and to dark. Eight of these have shown 1 or more of the following responses to light: variability in sporulation, sclerotial formation, sporodochial formation, zonation, pycnidial production, or pigmentation. Few or no sclerotia are produced by *Sclerotium rolfsii* in the absence of light, but they are formed abundantly when cultures are exposed to light. A strain of *Stemphylium* sp., 1 of *Pestalotia* sp., and 1 of *Verticillium albo-atrum* sporulate freely under fluorescent, mazda, or blue light but not under red or far red (about 7000A and above) light or in dark. On the other hand, *Monilinia fructicola* sporulates freely, *Endothia parasitica* pigments poorly, and Verticillium produces abundant microsclerotia under red or far red light or in the dark. A mutant Pestalotia sporulates freely under all light conditions tested. Pestalotia and Monilinia become light-sensitive following 2 days' growth on potato-dextrose agar. Seven hours of fluorescent light on the third day initiates abundant sporulation of Pestalotia, but good sporulation of Monilinia is initiated by 7 hours dark. Three hours dark is insufficient for sporulation of Monilinia, whereas 5 hours results in moderate sporulation.

Control of blackspot of roses with dust combinations containing fungicides, insecticides, and miticides. MCCLELLAN, W. D., FLOYD F. SMITH, AND EDGAR A. TAYLOR. In 1954, 29 dust combinations were tested on 5 varieties of roses in 120 field plots. Zineb was used alone or in combination with 1.5 per cent Aramite. All other fungicides were used alone or in combination with Aramite 1.5 per cent, Ovotran 1.5 per cent, or malathion 4 per cent. Yield differences due to fungicidal treatment were not significant for the blackspot-resistant variety Red Radiance, but they were statistically significant for the blackspot-susceptible varieties Chief Seattle and Golden Masterpiece. Both the number of flowers and the average weight per flower were reduced by blackspot (caused by *Diplocarpon rosae*). The mean numbers of Golden Masterpiece flowers cut during 1954 were as follows: no fungicide, 924; copper oxysulfate (3.4 per cent copper) with 65 per cent sulfur substituted in October and September, 907; Copper oxysulfate (3.4 per cent copper) and sulfur (25 per cent), 1147; ferbam (7.6 per cent) and sulfur (25 per cent), 1085; captan (5 per cent), 1107; Karathane (1 per cent), 909; and zineb (6.5 per cent), 1105. The comparable mean weights (g) of the flowers cut in September were 7.2, 7.0, 9.2, 10.1, 9.1, 7.5, and 9.8, respectively. In contrast to the adverse effects of captan on malathion, there were no interactions between the fungicides and miticides so far as blackspot control was concerned.

Reaction of various hosts to the fungus causing anthracnose of tobacco. MORGAN, O. D. Studies on the host range of the *Colletotrichum* sp. causing anthracnose of tobacco indicated that this organism will attack a wide variety of hosts. Of 48 cultivated and wild plants inoculated in the greenhouse, 23 were infected. The study on the host reaction to the fungus was made to determine possible sources

of the disease organism. On the basis of the reactions that occurred, the hosts could be divided into 3 major groups: 1) leaf-spot, stem, petiole, and vein-lesion group which contained most of the plants under study (tobacco, pepper, tomato, cucumber, soybean, jimson weed, garden pea, sugar beet, coleus, geranium, and begonia); 2) dry-rot group which included the grasses red top, brome, sudan, and rye (some leaf spots occurred on brome and sudan grass); and 3) wilt group with petiole, vein, and stem lesions occurred mostly on small plants. Included in group 3 were leaf and head lettuce, a species of wild lettuce, alfalfa, red and ladino clover, and sunflower. Results of the tests gave information on type of injury to recognize on crops and other plants growing in tobacco beds. Plants found naturally infected were tomato, jimson weed, leaf lettuce, wild lettuce, pepper, and 2 unidentified grasses.

Captan, zineb, and captan + zineb for the control of the fruit rot phase of Botryosphaeria ribis on apples. ROMANKO, R. R., AND J. W. HEUBERGER. Data obtained on fruit of the Rome variety in 1953, under conditions of light infection (11 per cent), indicated that captan and zineb might be effective in control. Accordingly, in 1954 a block of the Rome variety was used for a large-scale field experiment in which captan (1½–100), zineb (1½–100), and captan + zineb (¾–¾–100) in the period covered by the fourth through eighth cover spray applications. (June 7–August 23) were tested. Each material was applied with a speed sprayer to a 4-row block (88 trees). At harvest, rot counts were made on 250 fruits from each of 4 trees for each treatment. The percentage fruit infection was as follows: unsprayed, 37; captan, 6; zineb, 9; and captan + zineb, 7. Storage studies indicated that the control effect of the treatments carried over into storage. After storage at 1.5°C for 87 days after harvest, percentage fruit infection was as follows: unsprayed, 45; captan, 3; zineb, 8; and captan + zineb, 19.

Effect of some soil factors on efficiency of fungicides in controlling Rhizoctonia solani. RUSHDI, M. H. K., AND W. F. JEFFERS. In liquid medium, 4 ppm Puratized Agricultural Spray (6 per cent (tris(2-hydroxyethyl)(phenylmercuri)ammonium lactate) or 20 ppm Arasan (50 per cent thiram), Semesan (30 per cent 2-chloro-4-(hydroxymercuri)phenol), Vancide 51 (30 per cent total active ingredients: sodium dimethyldithiocarbamate and a sodium derivative of 2-mercaptobenzothiazole), or Zerlate (76 per cent ziram) caused complete inhibition of growth of *Rhizoctonia solani*. At 20 ppm, Fermate (76 per cent ferbam) and Acti-dione (90 per cent cycloheximide) caused 75 per cent inhibition and Dithane Z-78 (65 per cent zineb) was only slightly toxic. Copper compounds caused little reduction in growth. Effects of soil factors on growth of *R. solani* were studied in tubes containing a soil of normally high clay content. Increase in soil moisture and soil temperature did not affect fungicidal efficiency. Addition of 1 per cent starch reduced the activity of nabam and of Puratized Agricultural Spray as well as linear growth of the organism, whereas 1 per cent casein had no effect. Addition of 1 per cent hay or wheat straw reduced the efficiency of nabam and of Puratized Agricultural Spray. Five per cent hay reduced activity of all fungicides tested except Vancide 51. When the normal soil pH was raised from 4.5 to 6.1, activity of Vancide 51, nabam, and Puratized Agricultural Spray was increased whereas activity of Acti-dione was decreased. At pH 7.1 activity of Vancide 51 was increased; there was no effect on nabam and Puratized Agricultural Spray and activity of Acti-dione was further decreased.

Structure, function, and host in root-knot nematodes. STEINER, G. Sedentary endoparasitism in roots of plants affects structures and functions of root-knot nematodes in various ways. Observations show that root-knot nematodes inside roots take a position with the head in a proximal and the tail in a distal direction, with the ventral side toward the root surface and the dorsal side toward the vascular bundle; thus eggs are near to, and hatching larvae are released close to, the root surface and soil. This mode of establishment of the nematode is also accompanied by a dorsally curved buccal stylet in parasitic larvae and adults, a feature hitherto overlooked. The "cheeks" on the head of male root-knot nematodes represent ampullas of the amphid and are apparently structures to increase efficiency of this chemical-sense organ, which in the male is enlarged and complex to assure location of female. Torsion of body of male root-knot nematodes is to insure effective copulation. The filiform and cylindrical male thus may encircle the globose female effectively. Degree of torsion appears specific according to species; it may involve only 180°, but a maximum of 2½ turns (900°) has been observed.

The intake of solutions by tops of freshly cut oak stumps. TRUE, R. P., T. M. JUDY, AND ELDON ROSS. Several conventional silvicides produce a slow or incomplete root-kill in treated oaks. These are not entirely satisfactory for use in setting up underground barriers of poisoned root systems around oak wilt trees to prevent root-graft spread of the disease. Studies were undertaken to determine the volumes of water, of a water-soluble dye, and of a water-soluble poison that would be taken in through the surfaces of freshly cut stumps from stump-top reservoirs placed on red oak stumps cut in summer. Stumps took up about 1 liter of water per diameter-inch. Isolated stumps took up most of this volume in the first 24 hours. Comparable stumps absorbed less than ½ this volume of azosulfamide, a water-soluble dye, and less than 1/10 as much of a saturated solution of copper sulfate. The presence of standing live companion sprouts and of other oaks root-grafted to the treated tree greatly increased and prolonged absorption. Both the dye and the copper sulfate were translocated to the crowns of trees organically united to the treated stump.

Comparison of some methods of dormant conservation of fungus cultures. WEISS, FREEMAN A., AND SHUH-WEI HWANG. In the routine maintenance of a large number of cultures of microorganisms, a comparison has been made of lyophilization or freeze-drying, oil sealing of agar slants, and desiccation on soil or other inert substrates. A method, first suggested by J. Lederberg, of rapid desiccation of a spore suspension on granulated silica gel in small vials, which are then sealed without desiccation, has given a high rate of survival for 20 months. Use of this method has resulted in successful preservation of the following genera: *Alternaria, Aspergillus, Candida, Colletotrichum, Fusarium, Helminthosporium, Penicillium, Saccharomyces, Streptomyces, Torulopsis,* and *Trichoderma.*

A bacterium from beans antagonistic to Xanthomonas phaseoli and Corynebacterium flaccumfaciens. ZAUMEYER, W. J. A bacterium tentatively identified as *Bacterium herbicola* that showed antibiosis against a number of bacterial plant pathogens was isolated from bean leaves infected with *X. phaseoli* collected in Colorado and Nebraska in 1954. What appeared to be the same organism had been isolated previously from beans from these states. *B. herbicola* may be mildly parasitic on beans but no positive symptoms have been observed. It produced inhibition zones of varying widths when streaked on agar plates seeded with the following plant pathogens: *X. phaseoli, X. phaseoli* var. *sojense, X. pruni, X. ricinicola, Pseudomonas phaseolicola, P. lachrymans, P. sesami, P. glycinea, Corynebacterium flaccumfaciens, Erwinia carotovora,* and *E. atroseptica.* Beef-broth cultures of *X. phaseoli,* or *C. flaccumfaciens,* plus *B. herbicola* incubated for 7 days at 28°C produced no infection when used for the inoculation of greenhouse-grown beans, whereas the pathogens grown alone in broth produced typical infection. Cultures of these 2 pathogens grown alone for 2 days then seeded with *B. herbicola* and incubated for 5 days produced no infection on beans, whereas the pathogens grown alone in broth for the same period produced infection. When the same procedure was followed with *P. phaseolicola,* infection on beans was not inhibited but disease severity was reduced.

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GEO. H. CONANT, **RIPON, WISCONSIN**

Congratulations to E. E. Clayton who was awarded an honorary Doctor of Science Degree by the North Carolina State College during Commencement on May 29. Dr. Clayton, formerly in charge of tobacco breeding and disease work for the U.S.D.A., was cited for his outstanding contributions in tobacco breeding and disease research leading directly to the development of disease resistant varieties which have meant so much to growers and the economic welfare of North Carolina and other tobacco growing states. Disease resistant varieties which Dr. Clayton has been instrumental in developing, now planted on more than 250,000 acres in North Carolina alone, are returning millions of dollars in increased profits to growers each year. Without these improved tobacco varieties, profitable tobacco production on thousands of disease-infected farms would be impossible. On the evening of May 28, prior to Commencement, Dr. and Mrs. Clayton were entertained by friends and co-workers from Experiment Stations, industry and farm groups with a banquet on the North Carolina State College Campus.

After 25 years of service as Chairman of the Department of Plant Pathology at the University of Wisconsin, Dr. G. W. Keitt has requested that he be relieved of administrative duties. He will continue teaching and research. Dr. Glenn S. Pound has succeeded Dr. Keitt in the chairmanship.

The C. R. Orton Memorial Loan Fund is being established at West Virginia University in memory of Dr. Orton. The fund is being raised through contributions from his many friends and associates.

Plant Pathologist G. B. Sanford recently retired as Pathologist-in-charge, Science Service Laboratory, Edmonton, Alberta, Canada. He has been succeeded by Dr. L. E. Tyner.

The Canadian Phytopathological Society held its 18th Annual Meeting in conjunction with the Agricultural Institute of Canada, June 20-23 at the University of Alberta, Edmonton. Membership in the Canadian Society totals 232 and many of its members are also affiliated with our Society. Officers for 1955-56 are: W. F. Hanna, President; R. E. Fitzpatrick, Vice-President; D. S. MacLachlan, Secretary-Treasurer; W. C. Broadfoot and F. Godbout, Councilors. The 1956 meeting will be held in Toronto, Ontario.

Emeritus Professor of Plant Pathology is the new title for Walter S. Beach who retired July 1 after 37 years of service as Professor of Plant Pathology at Pennsylvania State University. Dr. Beach conducted research on diseases of vegetables, mushrooms, tobacco, and turf grasses.

Phytopathologist Otto A. Reinking, Foreign Agricultural Advisor, F.O.A. returned in March 1955 from the Philippines after a five month tour of the Abaca (Manila hemp) mosaic disease situation. Dr. Reinking was recalled to review the Abaca mosaic program and to evaluate the Abaca industry in relation to the disease and rehabilitation program. Since 1950 he has served as a consultant on the program and was given a Superior Service Award by the U. S. Department of Agriculture for his mosaic control campaign. Dr. Reinking retired from the Government Service in June.

Editor: K. W. KREITLOW, Plant Industry Station, Beltsville, Md.

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